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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXVII | *JANUARY, 1948* | NUMBER 1 CONSECUTIVE NO. 2

The Anesthetic Effects of Some New Barbituric Acid Derivatives Administered to Dogs. III*

By WILLIAM H. HUNT†, RUSSEL J. FOSBINDER, and O. W. BARLOW

A selected group of new barbituric and thiobarbituric acids have been studied by both oral and intravenous administration to dogs. Extensive data on induction time, stages and duration of anesthesia, effects on blood pressure, heart and respiratory rates, and urinary excretion are reported. Thiobarbiturates in the same dosage containing the R—S(CH$_3$)H— produced a greater duration of anesthesia than those containing the R—S.CH$_2$—group. The compounds studied are believed to be detoxified by the liver.

PRELIMINARY studies of approximately 150 new 5,5-disubstituted barbituric and thiobarbituric acid derivatives, described by Walter, Goodson, and Fosbinder (1), have been made in our laboratories. These studies (2, 3) revealed the onset and duration of anesthesia and acute toxicity of these compounds when administered intraperitoneally to mice, orally and intravenously to rats, and intravenously to rabbits. The results of these studies served to permit the selection of a group of compounds which seemed to merit more intensive investigation in the dog.

* Received Aug. 29, 1947, from the Research Laboratories of the Maltbie Chemical Co., Newark, N. J., and the Sterling-Winthrop Research Institute, Rensselaer, N. Y.

† Present address: Marvin R. Thompson, Inc., Stamford, Conn.

EXPERIMENTAL

A. Intravenous Administration.—A series of twelve male and female dogs were standardized by the administration of sodium salts of Evipal, pentobarbital, and Pentothal at doses considered to be optimal for producing a definite anesthetic action. In all cases preceding the intravenous administration of each compound, food was withheld sixteen to eighteen hours but water was permitted ad libitum. A seven-day interval was allowed between successive doses of the compounds to permit clearance of any residual barbiturate. Five per cent solutions of the sodium salts of the compounds under investigation were injected at the rate of 2 cc. per minute except PT-152, 166, and 192 which were injected at the rate of 1 cc., 1.5 cc., and 1.5 cc. per minute, respectively. The total dose of Evipal sodium was injected in two minutes.

The observations on each animal included:

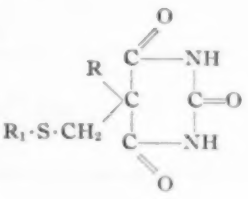
Onset of Anesthesia—intervals between injection and loss of pad reflex.
Respiratory Rate—observed at 10-minute intervals until the animal raised its head and assumed the belly position.
Down Time—onset of anesthesia until the animal stood.
Full Recovery—onset of anesthesia until the animal could climb stairs without ataxia.

Untoward effects were noted in three of the compounds under observation. PT-164 (30 mg./Kg.) produced mild struggling during three-fourths of the injection in three of the six animals observed. PT-114 (40 mg./Kg.) caused death by respiratory paralysis in two animals and severe respiratory embarrassment in a third which was relieved by artificial respiration in thirty minutes. A dose of 20 mg./Kg. only moderately decreased the respiratory rate. Seventy-five minutes after the administration of PT-192 (30 mg./Kg.) and during the respiratory phase in light anesthesia, 2 of 6 animals exhibited tremors of the leg muscles for a period of twenty-nine minutes.

In a comparison of the duration of anesthesia (Table I) it may be seen that PT-109 and PT-114 exhibited a longer duration of anesthesia than pentobarbital sodium while PT-164, 192, 199, 200, and 204 ranged between Evipal sodium and pentobarbital sodium and PT-68, 125, 152, and 166 were of shorter duration than Pentothal sodium. It is evident that the selected compounds of the new series are representative of all types of barbituric acids ranging from the long to the ultra-short acting anesthetics.

In the clinical application of an intravenous barbiturate, the effect upon respiratory rate is an important consideration. A review of the average lowest respiratory rate (Table I) reveals that only PT-68 and PT-125 decreased the rate more than pentobarbital sodium which was the most depressing reference compound (PT-85 was not considered because of the high dosage necessary to produce anesthesia).

The compounds referred to in Table I are as follows:



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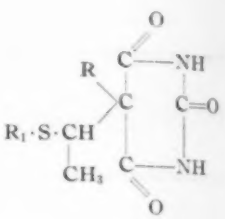

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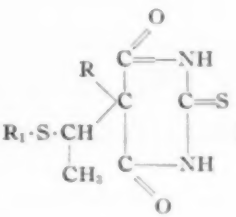

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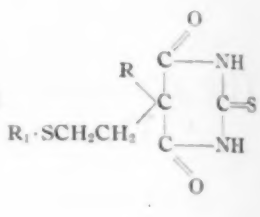

IV

TABLE I.—EFFECTS OF VARIOUS SUBSTITUTED BARBITURIC AND THIOBARBITURIC ACIDS INJECTED INTRAVENOUSLY IN DOGS

Compound		Reference No.	No. of Animals	Dose, Mg./Kg.	Duration of Anesthesia, Min.	Down Time, Min.	Return to Normal, Min.	Average Lowest Respiratory Rate
Evipal Sodium		...	12	40	69	183	340	19
		...	4	35	259	291	368	8
Pentobarbital sodium		...	8	25	200	252	358	11
Pentothal sodium		...	12	26.25	50	69	137	15
Compound I								
R	R_1							
1-Methyl butyl	Ethyl	68	6	40	188	241	336	6[a]
		...	6	30	166	243	348	10
		...	3	20	37	57	196	28
Isobutyl	Methyl	85	6	80	244	290	405	7
1-Methyl butyl	Allyl	164	6	30	118	155	253	11
1-Methyl butyl	Methyl	192	6	30	92	118	159	11
Compound II								
Ethyl	Butyl	109	6	30	280	377	506	11
Isobutyl	Allyl	125	1	35	145	306	527	4
		...	6	25	46	58	151	16
Allyl	Butyl	114	6	40	422	672	742	9
		...	3	20	258	343	478	8
Allyl	Ethyl	199	5	40	85	121	178	14
Allyl	Allyl	200	6	30	157	274	369	10
Methyllyl	Allyl	204	4	25	136	217	294	10
Compound III								
Ethyl	Ethyl	166	6	30	37	47	75	15
Compound IV								
Allyl	Butyl	152	6	30	37	43	72	18

[a]Salivation observed in all animals.

TABLE II.—EFFECTS OF VARIOUS SUBSTITUTED BARBITURIC AND THIOBARBITURIC ACIDS ADMINISTERED ORALLY TO DOGS

Compound	Number of Animals	Dose, Mg./Kg.	Duration of Loss of Various Reflexes, Min.			Induction, Min.	Down Time to Normal	
			Toe Pad	Skin	Ear Twist		Hr.	Min.
PT-164	3	20	35	55	58	11	4	54
	2	30	+	+	+	32	4	55
	3	40	+	19	39	3	3	11
	3	60	142	225	225	9	12	5
PT-109	2	20	4	19	19	15	3	57
	3	30	40	38	57	15	6	25
	2	40	75	90	112	7	9	00
	1	60	270	318	348	8	22	12
PT-114	6	20	20	51	53	10	8	44
	4	30	38	126	159	9	14	18
	2	40	165	195	270	6	8	58
PT-125	1	20	+	+	+	+	+	+
	2	30	+	+	+	+	3	51
	4	40	35	40	97	34	9	33
	3	50	4	14	121	8	11	11
	1	60	250	310	370	8	>18	00
PT-199	5	20	12	12	18	11	3	25
	5	40	44	61	33	23	3	18
	5	60	85	127	89	18	4	29
PT-166	1	30	+	+	+	+	3	40
	2	40	+	5	5	3	2	52
	5	60	6	77	104	14	6	42
	2	80	50	144	181	10	>7	00
	1	90	52	67	82	36	6	07
PT-152	2	20	+	+	+	+	2	13
	2	30	+	8	+	6	1	29
	1	40	+	+	+	24	6	00
	5	60	+	16	16	4	4	19
	4	90	49	60	71	4	3	51

It is apparent from these results (Table I) that the majority of the new compounds are relatively free from producing dangerously low respiratory rates following intravenous administration.

The results of the intravenous study aided in the selection of five barbituric (PT-109, 114, 125, 164, and 199) and two thiobarbituric (PT-152 and 155) acids, which were deemed interesting enough to warrant further investigation. These compounds were studied intravenously in dogs of mixed sex for their effect on blood pressure and on heart and respiratory rates. Under ether anesthesia a carotid artery was cannulated, an airway inserted in the tracheae and a saphenous vein exposed for intravenous injections. After preparation of the animals very light ether anesthesia was maintained since doses of barbiturates, equivalent to those employed in the previous intravenous studies, when administered during medium to light anesthesia, produced death by respiratory failure. Blood pressures were registered by a mercury manometer and respiratory movements were recorded directly from an area of skin slightly below the diaphragm.

Sodium salts of PT-109, 114, 125, 164, 199, 152, and 166 were prepared as 2.5% solutions and injected intravenously. Initial injections were made at the rate of 1 cc. per minute with the exception of PT-109 (30 mg./Kg. at 2 cc. per minute, 15 and 20 mg./Kg. at 0.5 cc. per minute) and PT-125 which was administered at 0.5 cc. per minute. Any alteration in the rate of injection of successive doses of any one compound is indicated in the text below.

Following the administration of the above mentioned barbiturates, vagal stimulation with induced current did not produce any alteration in heart rate or blood pressure.

Three dogs received initially the intravenous administration of 15, 20, and 30 mg./Kg. of PT-109, respectively. Subsequent doses of 15 mg./Kg. in the first animal decreased the respirations more than did successive doses of 20 mg./Kg. in the second animal and 10 mg./Kg. in the third. Also subsequent 20 mg./Kg. doses failed to produce more than a medium surgical anesthesia in contrast to the heavy stage reached with successive doses of 15 and 10 mg./Kg. in the first and third animals, respectively. The 30 mg./Kg. dose injected at an increased rate (2 cc. per minute) sustained a definitely depressed blood pressure and decreased heart rate even after the appearance of light surgical anesthesia.

Two animals were initially injected with PT-114 (10 mg./Kg.). Medium surgical anesthesia was maintained with difficulty in one of the animals with four successive doses of this amount and the animal succumbed from respiratory failure following the fourth injection. In the second dog heavy surgical anesthesia was obtained after the first injection. A second administration of 5 mg./Kg. decreased the heart rate, blood pressure, and respirations slightly and only medium surgical anesthesia ensued. A third injection of 10 mg./Kg. deepened the anesthesia but severely decreased the respiratory rate and produced cardiac arrhythmia.

TABLE III.—URINARY EXCRETION OF BARBITURIC AND THIOBARBITURIC ACIDS ADMINISTERED ORALLY TO DOGS

Compound	Weight of Dog, Kg.	Dose, Mg./Kg.	Stage of Anesthesia	Cumulative Recovery in Urine of Administered Dose		
				Hr.	Mg.	%
PT-109	16.8	30	Heavy	1	19.0	...
			Medium to light	2	47.5	...
			.....	24	61.0	12.09
	13.2	30	Light	1	14.0	...
			.....	2	30.0	...
			.....	24	83.0	20.97
PT-114	17.3	20	Light	1	5.7	...
			.....	2	11.2	...
			.....	5	11.2	3.25
	13.4	30	Heavy	1	4.6	...
			Medium to light	2	12.8	...
			.....	5	28.5	7.08
PT-125	13.0	40	Not completely	1	2.5	...
			anesthetized	2	4.3	...
			.....	5	4.3	0.83
	11.9	50	Light	1	3.6	...
			.....	2	11.6	...
			.....	5	16.9	2.84
PT-199	17.2	40	Heavy	1	15.0	...
			Medium	2	21.2	...
			.....	24	82.2	11.95
	14.1	40	Very light	1	0.0	...
			.....	2	19.6	...
			.....	24	76.6	13.59
	16.1	60	Heavy	1	7.2	...
			Medium	2	16.8	...
			.....	24	96.8	10.02
	11.4	60	Not anesthetized	1	6.8	...
			.....	2	6.8	...
			Possible retention	24	117.8	17.22
PT-164	12.8	30	Not anesthetized	1	6.1	...
			.....	2	13.7	...
			.....	24	95.2	24.82
	11.0	30	Light	1	18.0	...
			.....	2	22.9	...
			.....	24	91.9	27.86
PT-166	15.6	60	Light	1	20.6	...
			.....	2	37.1	...
			.....	24	112.1	11.97
	11.2	60	Light	1	15.5	...
			.....	2	28.5	...
			.....	24	116.5	17.34
PT-152	16.0	40	Not anesthetized	1	8.7	...
			.....	2	26.5	...
			.....	5	39.7	6.20
	11.0	60	Not down	1	13.2	...
			.....	2	18.1	...
			.....	5	36.1	5.47

Two animals each received four successive intravenous injections of 20 mg./Kg. of PT-125. After the second injection in one animal the anesthesia was medium in depth, whereas in the second it was heavy surgical. Both animals exhibited a slight to moderately decreased blood pressure and heart rate. Cardiac arrhythmia was evident in both animals after the third injection and death from respiratory failure followed the fourth injection.

Following two intravenous injections of 20 mg./Kg. of PT-164 heavy surgical anesthesia ensued with a slight transitory decrease in heart rate and blood pressure. Since a third injection at a rate of 2 cc. per minute resulted in desirable anesthetic properties without serious untoward effects, the injection rate was increased on the fourth injection to 4 cc. per minute to observe the effect on respiration. The respiratory rate was decreased to eight per minute and a fifth injection at 8 cc. per minute produced death by respiratory paralysis.

Four successive doses of 25 mg./Kg. of PT-199 were injected intravenously in two dogs. Heavy surgical anesthesia was obtained. The respiratory rate was considerably slowed and a sustained fall in blood pressure was noted. In one animal the fourth injection was administered at the rate of 2 cc. per minute and death ensued immediately. In the second animal the fourth injection was administered at the previous rate of 1 cc. per minute and death occurred in ten minutes from respiratory paralysis.

The intravenous administration of 20 mg./Kg.

of PT-152 to two dogs produced a transitory decrease in respiratory rate immediately after each injection. In one animal four successive doses within seven minutes were required to produce heavy surgical anesthesia. The second animal received the third and fourth successive injections at an increased rate of 2 cc. per minute without obtaining heavy surgical anesthesia. A fifth injection two minutes later of 40 mg./Kg. resulted in heavy surgical anesthesia. None of the injections in either animal produced respiratory embarrassment or other untoward effects.

The intravenous administration of 20 mg./Kg. of PT-166 to two animals revealed that it was necessary to administer three doses at approximately fifteen-minute intervals to produce heavy surgical anesthesia. Other than the transitory fall in pressure produced by the initial dose no undesirable reactions were noted. A sustained moderate slowing of respiration was the only effect noted following the fourth dose. One animal succumbed from respiratory paralysis while the other survived the sixth dose.

The initial doses of the barbituric acids employed in this study were capable of inducing heavy surgical anesthesia, while the thiobarbituric acids required multiple doses to induce this stage of anesthesia. The dosages administered had little or no effect on heart rate except in the case of PT-125 which produced a moderate slowing. Blood pressure was generally unaffected, being but moderately depressed by PT-125 and PT-166. The respiratory rate was moderately decreased by PT-114, 125, and 164, severely depressed (less than 10 per minute) by PT-109 and 199, but practically unaffected by the thiobarbituric acids, even when the multiple doses necessary for heavy surgical anesthesia were employed. The cardiac arrhythmia produced by some of these compounds was purposely induced by excessive dosage. When optimal doses and rates of administration were maintained with these compounds objectionable reactions were not observed.

B. Oral Administration.—Seven of the new compounds (PT-164, 109, 114, 125, 199, 166, 152) were selected for oral administration and observations were made of the onset, stages, and duration of anesthesia. The compounds were administered by stomach tube as 5% solutions of the sodium salts.

The results following the oral administration of graded doses, tabulated in Table II, reveal that the selected compounds are capable of producing an anesthesia ranging from short to long duration.

Urinary excretion levels were obtained following catheterization of trained dogs. These observations were made at periodic intervals, as shown in Table III. Simultaneous blood level observations were made but abandoned when it was found that the present available methods, while satisfactory for urine determinations, were insufficiently sensitive for accurate measurement, due to the small quantity of compound recovered.

A review of the duration of anesthesia and urine excretion indicates that the duration was inversely proportional to the rate of excretion. However, PT-152 was an exception since it produced a very short duration of anesthesia with a low rate of excretion probably due to rapid destruction in the body, presumably by the liver; detoxification studies (3) conducted on the rabbit supported this theory.

SUMMARY

1. A group of new barbituric and thiobarbituric acids have been studied by intravenous and oral administration in dogs. Evipal sodium, pentobarbital sodium and Pentothal sodium were employed as reference compounds.

2. The induction time, stages and duration of anesthesia as produced by these compounds have been determined and observations on blood pressure, heart and respiratory rates and urinary excretion have been described.

3. A comparison of the anesthetic activity of the intravenously or orally administered barbituric acids, at the same dosage, reveals that those compounds containing the R—S·CH(CH$_3$)— group resulted in a greater duration of anesthesia than those containing the R—S·CH$_2$— group. The greater duration of anesthesia is believed to be due to the slower urinary excretion of the compound.

4. The very short duration of anesthesia observed following the administration of the thiobarbiturates indicates rapid detoxification of these compounds in the body, presumably by the liver.

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(2) Hunt, W. H., Fosbinder, R. J., and Barlow, O. W., THIS JOURNAL, **35**, 231–43(1946).
(3) *Ibid.*, 244–49.

Variations in the Standardization of Iodine Solutions for Analytical Purposes*

By C. K. BANKS†

The official methods require that iodine be standardized against arsenic trioxide and sodium thiosulfate against potassium dichromate. Theoretically, such thiosulfate and iodine solutions should be and frequently are equivalent. However, in a sufficient number of instances it was noted that the correspondence was not unity. Investigation indicated that such iodine solutions when first acidified, then made neutral and standardized against arsenic trioxide, showed no variation when compared with standard thiosulfate. The difficulty was found to be in the storage container and it is postulated that iodate formation is responsible for the variation noted. Examples of discrepancies in analysis as a result of variation were demonstrated. It is recommended that additional precautions be observed in the storage of iodine solutions and that either iodine solutions be required to show no variation in standardization or that a double standard be established, depending upon the particular conditions of use intended.

ON OCCASION iodine solutions have been found to possess an unexpected property: that of not exhibiting the same normality when compared with sodium arsenite as when compared with sodium thiosulfate. Since official procedures require that iodine be standardized against arsenic trioxide and thiosulfate against dichromate, this may lead to serious errors when both iodine and thiosulfate are used in the same procedure. Also, it raises the question as to whether the normality of the iodine solution as obtained by arsenite standardization is the proper value for all of the analytical uses of iodine. The theory of iodimetry and iodometry is based on the assumption that the potential of the iodine-iodide half-cell is independent of the hydrogen-ion concentration. The use of standard iodine solutions for volumetric determinations has been thoroughly investigated and it is extremely unlikely that variation from theoretical behavior would have been overlooked. While many iodine solutions exhibited no abnormalities when first examined, they frequently showed variations on standing. Considering the possible errors involved in the standardizations, the differences were scarcely significant, but an occasional iodine solution exhibited a difference in normality sufficiently great as to be definitely outside the allowable experimental variation. As the iodine solutions were being used for the investigation of the purity of a number of compounds, we were aware that such variations might invalidate our findings. Also, any such variation in normality might have appreciable effect on official pharmaceutical assay procedures. It seemed of particular importance to locate the cause and study the effects of such variations.

An aged iodine solution showing a significant discrepancy in standardization was available. The complete past history of this solution was known, and sufficient iodine crystals of the original lot were available to prepare fresh solutions. To reduce cumulative errors, standard sodium-arsenite solution (from B. of S. arsenic trioxide) and standard sodium thiosulfate (by dichromate) were both compared with a standard potassium-bromate solution.

The total cumulative error between the thiosulfate and arsenite proved to be less than five parts in ten thousand. A freshly prepared iodine solution (in Pyrex glass) was then titrated with both arsenite and thiosulfate. As expected, the normalities obtained compared within one part per thousand, less than the allowable experimental error. As a further check, iodine samples were first carefully acidified and then buffered to neutrality and titrated with arsenite. The value so obtained compared within the allowable experimental error.

* Received Sept. 25, 1947, from the Research Laboratories, Parke, Davis and Co., Detroit, Mich.
Presented to the Scientific Section, A. PH. A. Convention, Milwaukee, Wis., August, 1947.
† The author wishes to thank Mr. F. A. Maurina of our Analytical Laboratories for many helpful suggestions and Mrs. J. D. Mitulski for technical assistance.

The aged iodine was examined in a similar fashion with markedly different results. While the thiosulfate value and that obtained by first acidifying the iodine and then titrating with arsenite in buffered solution agreed within two parts per thousand, direct titration of arsenite with iodine gave a value lower than this by six parts per thousand. This value might conceivably be an experimental error, but the magnitude of the variation is greater than the precision involved. It would seem that in such a solution there is iodine available to thiosulfate but not to neutral arsenite. The release of this iodine on acidification is suggestive of the presence of iodate. Since the *p*H of the aged iodine solution was 7.8 as contrasted to 5.8 for fresh iodine, the effect of containers on the *p*H of the solution and the iodine value was investigated.

Freshly prepared iodine in Pyrex glass showed no change on standing but when stored in a new amber-colored soft-glass bottle, the *p*H increased rapidly, changing from the characteristic 5 to 6 of freshly prepared solutions to 7 in four days and 8.6 in two weeks. Simultaneously, the normality of the iodine decreased as compared to arsenite but remained relatively constant as compared to thiosulfate. This solution showed a spread in standardization of sixteen parts per thousand at the end of two weeks. This difference is unquestionably significant.

As a further check upon the hypothesis that the container was responsible for the variation, amber bottles were washed with commercial soap powders, then rinsed nine times with distilled water. Water placed in such bottles became alkaline (*p*H 8) overnight. If the bottles were rinsed with acetic acid following washing and then subsequently with distilled water, the alkaline shift did not occur immediately but was just as great within a week. Pyrex glass did not impart an alkalinity to water or to iodine solutions.

To determine the possible error that might be introduced by improper standardization, oxophenarsine hydrochloride, sodium arsenite, ascorbic acid, sodium thiosulfate, and a new arsenical, oxophenarsine triazine [(trihydroxy)4-(2,4-diamino-6-*s*-triazinyl)-ammoniumbenzenearsonate (III)] (1), were titrated with iodine solution III by U. S. P. XII and N. F. VIII procedures. The results are given in Table III, calculated on the basis of arsenite and thiosulfate standardizations.

EXPERIMENTAL

All standard solutions were prepared in calibrated volumetric equipment. All chemicals were of the best commercial grades. The burettes used for comparison purposes were weight calibrated. Unless otherwise noted the solutions and procedures are from Kolthoff and Sandell (2). The values in Tables I and II have been converted to a standard volume of one reagent at exactly 0.05000 *N* for the purpose of ready comparison.

TABLE I.—EQUIVALENCE OF STANDARD SOLUTIONS (All Solutions Calculated as 0.05000 *N*. Volume in Ml.)

$KBrO_3$	As_2O_3	$Na_2S_2O_3$
40.00	40.01	39.95
40.00	39.99	40.03
40.00	39.99	40.00
40.00	40.00	39.99
Average	40.00 ± 0.01	39.99 ± 0.04
N(X)/N($KBrO_3$)	1.0001 ± 0.0002	1.0004 ± 0.0005

Solutions

Sodium Arsenite, 0.05000 *N*, from Bureau of Standards arsenic trioxide.

Potassium Bromate, 0.05000 *N* from reagent grade crystals.

Sodium Thiosulfate, 0.04970 *N*, standardized by potassium dichromate.

Iodine Solution—I, ca. 0.05 *N*, freshly prepared from chlorine-free, resublimed iodine, kept in Pyrex containers.

Iodine Solution—II, ca. 0.05 *N*, eighteen months old, stored in amber soft-glass bottle.

Iodine Solution—III, ca. 0.05 *N*, freshly prepared, stored in amber soft-glass bottle.

Comparison of Standards

Bromate-Arsenite.—Titrated at 90° in 80 ml. 6 *N* sulfuric acid, end point determined electrometrically using platinum and calomel electrodes. Values reduced to a standard bromate volume of 40.00 ml.

Bromate-Thiosulfate (2).—Values of thiosulfate reduced to 0.05000 *N* equivalence by dichromate.

Titrations of Iodine Solutions

A. Iodine-Thiosulfate (2).—Values converted to 40.000 ml. iodine of 0.05000 *N* equivalence.

B. Iodine-Arsenite (2).—Values converted to 40.000 ml. iodine of 0.05000 *N* equivalence.

TABLE II.—STANDARDIZATION OF IODINE SOLUTIONS

(All Solutions Calculated as 0.05000 *N* with Respect to Thiosulfate. Volume in Ml. Compared to 40 Ml. of Iodine Solution)

Iodine Solution: Identification	Age	pH	(A) Thiosulfate (Acidified W/Acetic Acid)	(B) Arsenic Trioxide (Neutral Buffer)	(C) Arsenic Trioxide (Iodine Acidified W/Acetic Acid, Buffered Neutral)
I	<7 days (Pyrex)	5.8	40.01 40.02 39.99 39.98 Av. = 40.000 ± 0.02 N = 0.05000 ± 0.00002	40.01 39.99 39.98 40.01 Av. = 39.998 ± 0.02 N = 0.05000 ± 0.00002	39.98 40.01 40.00 39.99 Av. = 39.995 ± 0.02 N = 0.04999 ± 0.00002
II	18 months (amber, soft glass)	7.8	39.97 39.99 40.02 40.03 Av. = 40.003 ± 0.03 N = 0.05000 ± 0.00003	39.76 39.78 39.75 39.73 Av. = 39.755 ± 0.03 N = 0.04969 ± 0.00003	39.96 40.01 39.99 39.96 Av. = 39.980 ± 0.04 N = 0.04997 ± 0.00005
III	1 day (Pyrex)	5.5	39.98 39.98 40.01 40.03 Av. = 40.000 ± 0.04 N = 0.05000 ± 0.00004	39.99 39.97 39.99 39.96 Av. = 39.977 ± 0.02 N = 0.04997 ± 0.00003	39.97 40.01 39.99 39.97 Av. = 39.985 ± 0.03 N = 0.04998 ± 0.00003
(transferred to amber soft-glass bottle)	2 days	6.2	40.02 40.00 39.96 40.00 Av. = 39.995 ± 0.035 N = 0.04999 ± 0.00004	40.00 39.98 39.98 39.96 Av. = 39.980 ± 0.02 N = 0.04998 ± 0.00002	....
	4 days	7.1	39.96 39.97 40.01 40.00 Av. = 39.985 ± 0.025 N = 0.04998 ± 0.00003	39.97 39.95 39.96 39.94 Av. = 39.955 ± 0.02 N = 0.04994 ± 0.00002	....
	7 days	8.2	40.01 40.00 40.02 39.98 Av. = 40.003 ± 0.02 N = 0.05000 ± 0.00002	39.61 39.58 39.58 39.55 Av. = 39.580 ± 0.03 N = 0.04948 ± 0.00003	40.00 39.96 39.97 39.96 Av. = 39.973 ± 0.03 N = 0.04997 ± 0.00003
	14 days	8.6	39.96 39.96 39.97 39.96 Av. = 39.963 ± 0.02 N = 0.04995 ± 0.00002	39.35 39.30 39.31 39.30 Av. = 39.315 ± 0.035 N = 0.04915 ± 0.00004	39.98 39.97 39.98 39.94 Av. = 39.968 ± 0.03 N = 0.04996 ± 0.00003

C. Iodine-Arsenite.—Iodine solutions samples (40 ml.) in iodine flasks were acidified with 0.5 ml. of concentrated hydrochloric acid, after five minutes brought to neutrality with borate buffer and titrated with the standard sodium arsenite solution The values were converted to 40.000 ml. of 0.05000 *N* iodine based on thiosulfate value.

DISCUSSION

From the data it is apparent that no principles of stoichiometry are at variance. The practical implications to pharmaceutical assays, however, are considerable. Errors of the magnitude encountered will greatly affect a number of assay procedures. A review of analytical textbooks and the literature has

shown that this factor has not received much consideration, probably due to several factors: (*a*) iodine solutions for analytical researches are generally freshly prepared; (*b*) they are frequently standardized under conditions *identical* with their use; and (*c*) student analytical procedures follow an established pattern. In routine analytical determinations iodine solution is prepared in large quantities, standardized against one standard, used under conditions not necessarily identical with the standardization, and frequently kept in amber soft-glass bottles. Most of the source books consulted recommended amber bottles for iodine, a factor conducive to error.

TABLE III

Product	Theory, %	Limits, %	Found I_2 = 0.04915 by Arsenite	Found I_2 = 0.04995 by Thiosulfate
Ascorbic Acid	100	>99	97.99	99.48
Oxophenarsine Hydrochloride[a]	31.81 As	30–32	30.90	31.37
Sodium Thiosulfate	100	>99	97.73	99.22
Arsenic Trioxide[b]	100	>99.8	99.91	101.51
Oxophenarsine Triazine[c]	22.82 As		22.52	22.86

[a] Sample contained 31.54% As by total combustion.
[b] Bureau of Standards arsenic trioxide.
[c] Sample contained 22.84% As by total combustion. This product can be obtained analytically pure by repeated recrystallization from water.

TABLE IV

Product	Error if an Iodine Solution Containing Iodate Is Standardized according to U. S. P. XII: U. S. P. XII	N. F. VIII
Acetone	Low	Low
Ascorbic Acid	Low	...
Arsenic Triiodide	None	None
Arsenic Trioxide	None	None
Arsenic and Mercuric Iodides	...	None
Iron Cacodylate	...	None
Mercurous Chloride	Low	Low
Mercurous Iodide	Low	Low
Methionine Chloride	Low	...
Oxophenarsine Hydrochloride	Low	...
Dichlorophenarsine Hydrochloride	Low	...
Potassium Antimony Tartrate	None	...
Propylene Glycol	...	None
Stibophen	...	Low
Sodium Bisulfite	Low	...
Sodium Thiosulfate	Low	Low
Sodium Cacodylate	None	None

From Table III, it can readily be seen that the arsenite standardization (official for U. S. P. XII and N. F. VIII) of iodine can lead to rejection of acceptable material. Iodine solution 3 is admittedly the worst encountered by us but even iodine solution 2 could cause a variation of over 0.5% in the assay of ascorbic acid. The calculated effect on a number of official procedures is given in Table IV. Not only are the procedures for determination of pharmaceutical products affected by variation in iodine standardization, but also many other routine analytical procedures. The determination of organically bound trivalent arsenic (3) is dependent upon acid-iodine titration of the organic compound. In this instance, standardization of iodine by B. of S. arsenic trioxide leads to appreciable error if the iodine solution contains iodate.

Further error may be introduced if iodate-containing iodine solutions, standardized against arsenite, are used to standardize thiosulfate solutions.

Theoretically, the development of iodate in iodide-iodine solution has not received much consideration (4). Washburn (5, 6) calculated that the *p*H of the arsenite-iodine titration should not exceed 9 to prevent iodate formation. The conditions in a 0.05 *N* iodine solution are such that using the equation developed by Washburn

$$\frac{[IO_3^-][I^-]^5}{[OH^-]^6[I_3^-]^3} = 6 \times 10^{28}$$

and assuming $[I_3^-] = 2.5 \times 10^{-2}$, $[I^-] = 0.1$, and $[OH^-]\,[H^+] = 10^{-14}$, the ion concentration of iodate at *p*H 7 would be of the order of 10^{-18}, at *p*H 8 it would be 10^{-4}, and at *p*H 9, 10^{3}. From this, it seems logical that iodate has developed in iodine solutions having an alkaline *p*H.

The general inferences are obvious; either iodine solutions should be standardized under conditions of acidity appropriate for the use to which they will be put, or several rigorous criteria should be established to prevent and detect the formation of iodate in iodine solutions. The principal criteria to be observed for good stoichiometry are: (*a*) all reagents used in preparing standard iodine solutions should be iodate free, (*b*) the *p*H of the solution when prepared should be less than 6 and remain less than 7 in use, (*c*) iodine solutions should be stored in Pyrex in the dark; amber or other soft-glass bottles should *not* be used, (*d*) any iodine solution developing a *p*H of over 7 should be discarded.

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The Colorimetric Determination of Homatropine Methylbromide*

By F. J. BANDELIN

Homatropine Methylbromide may be precipitated quantitatively as the insoluble Reineckate with Ammonium Reineckate. The precipitate is filtered off, washed, dissolved in acetone, and determined spectrophotometrically at 525 mμ. Necessary equipment and procedure employed are described and some analytical data given. A method for the extraction of Homatropine Methylbromide from tablets in preparation for determination and a discussion of interfering substances are also presented.

THE apparent widespread use and increased importance of Homatropine Methylbromide as an antispasmodic resulting from its inclusion in the N. F. VIII emphasizes the desirability of a rapid and simple method for the determination of this compound in pharmaceutical preparations.

Since both heterocyclic nitrogen compounds and quaternary ammonium salts form compounds of low solubility with Ammonium Reineckate, and since Homatropine Methylbromide may be regarded as both a heterocyclic nitrogen compound and a quaternary ammonium salt, the use of Ammonium Reineckate as a precipitant was considered. Preliminary investigations indicated that Homatropine Methylbromide could be quantitatively precipitated from aqueous solutions with Ammonium Reineckate and that, as the Reineckate, it could be filtered off, washed with water, then dissolved in acetone as in the method proposed by Beattie (1) for the colorimetric determination of choline. The colored acetone solution could then be determined colorimetrically.

EXPERIMENTAL

Reagents

(1) **Ammonium Reineckate Solution.**—Two grams of Ammonium Reineckate[1] was stirred with 100 cc. of distilled water for ten minutes using a mechanical stirrer. The resulting solution was filtered through a hard filter paper.

This solution decomposes on standing. It should be kept refrigerated and used not longer than forty-eight hours after preparation.

(2) **Sulfuric Acid, 20%.**

(3) **Standard Homatropine Methylbromide Solution.**—Dissolve 100 mg. of Homatropine Methylbromide in enough distilled water in a 50-cc. volumetric flask to make up to volume. Each cc. of this solution contains 2.0 mg. of Homatropine Methylbromide.

Preparation of Standard Curve

Pipette accurately 1, 2, 3, 4, and 5 cc., respectively, of the standard Homatropine Methylbromide solution into each of five test tubes. The volume in each tube is adjusted to approximately 10 cc. with distilled water and 1 cc. of the 20% sulfuric acid is added. Following this, 2 cc. of the Ammonium Reineckate solution is added to each of the test tubes which are then agitated and allowed to stand for one hour to assure complete precipitation. The precipitates are filtered through 15-cc. low-form, fritted-glass crucibles of medium porosity using suction and each is washed with two 2-cc. portions of distilled water. Suction is applied until all excess water has been drawn out of the precipitates. The precipitates are then dissolved in acetone by passing 2-cc. portions of acetone through the crucibles with the aid of gentle suction. The colored acetone solutions are collected in individual test tubes placed inside the suction flask and under the stem of the crucible holder or adapter. After four 2-cc. portions of acetone have been passed through the crucibles (all the precipitates usually dissolve in the first portion), the solutions are transferred to 10-cc. volumetric flasks using the remainder of the acetone to wash the test tubes and to bring the volume to 10 cc.

Spectral transmittance of the Homatropine Methylbromide Reineckate in acetone was determined and plotted in the range of 350 to 750 mμ using a Coleman Spectrophotometer, Model 14. The resulting curve indicated a peak absorption at 525 mμ. Ammonium Reineckate in acetone gave a similar curve with a peak of absorption at the same point (Fig. 1).

Percentage transmittance of the acetone solutions containing 2-mg. increments of the Homatropine Methylbromide Reineckate in the range of 2 to 10 mg. was therefore determined at a wave length of

[1] Eastman Kodak Company.

* Received Sept. 30, 1947, from the Research Laboratories, Flint, Eaton and Company, Decatur, Ill.
Presented to the Scientific Section, A. PH. A., Milwaukee meeting, August, 1947.

525 mμ using the Coleman Spectrophotometer.[2] The following typical set of values was obtained:

Concentration, Mg.	Transmission, %
2	85.5
4	73.2
6	62.5
8	53.9
10	46.2

These values when plotted on semilog paper were essentially a straight line in conformity with Beer's law (Fig. 2).

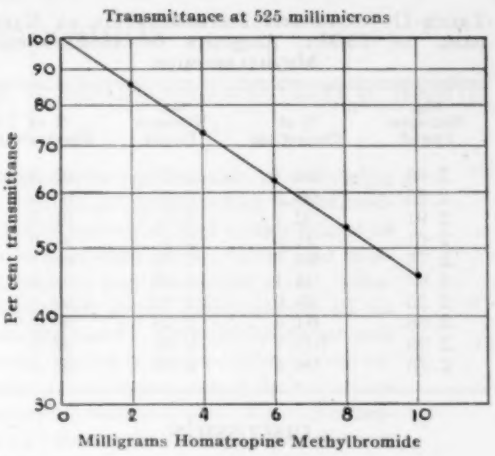

Figure 2

Determination in Tablets

An adequate number of tablets, usually 20 or more, to assure a representative sample, were ground to a fine powder and an aliquot equivalent to 5 mg. of Homatropine Methylbromide was accurately weighed. The sample was extracted with 5 cc. of distilled water by allowing to stand in a test tube with shaking at intervals. After about thirty minutes this mixture was filtered through a 15-cc. fritted glass crucible of medium porosity and washed with five 1-cc. portions of distilled water using suction. One cubic centimeter of 20% sulfuric acid was added to the combined filtrate and washings and this was followed by 2 cc. of Ammonium Reineckate solution. After allowing to stand, the Reineckate was filtered off, washed, and the acetone solution prepared for photometry according to the method given under the preparation of the standard curve.

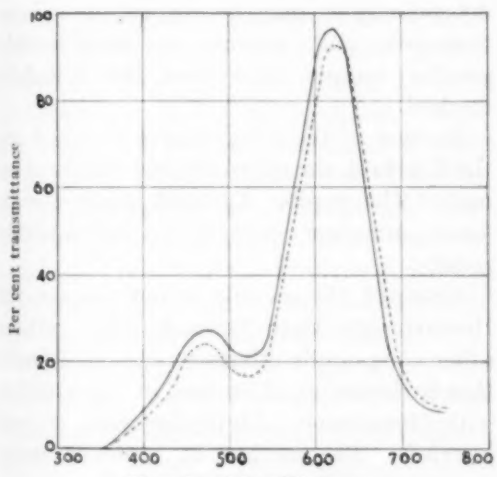

Figure 1

Results of 20 determinations on such aliquots from the same batch of tablets are given in the following table:

[2] Any suitable photoelectric colorimeter may be used.

TABLE I.—RECOVERY OF HOMATROPINE METHYLBROMIDE FROM 5-MG. ALIQUOTS

HMB Found	% of Theoretical	HMB Found	% of Theoretical
4.86	97.2	4.90	98.0
4.78	95.6	4.78	95.6
4.82	96.4	4.88	97.6
4.96	99.2	4.76	95.2
4.84	96.8	4.82	96.4
4.84	96.8	4.92	98.4
4.74	94.8	4.58	91.6
4.80	96.0	4.84	96.8
4.88	97.6	4.78	95.6
4.82	96.4	4.88	97.6

Nitrogen determinations were carried out by the Kjeldahl method on aliquots of powder containing 75 mg. of Homatropine Methylbromide. It was found not possible to use the micro-Kjeldahl method as recommended in the N. F. VIII monograph for tablets since an equivalent of thirty 2.5-mg. tablets are required to supply a 75-mg. sample. The ordinary 2.5-mg. tablet usually weighs about 1 gr. (65 mg.), a total of about 30 gr. (2.0 Gm.) for thirty tablets. Since the N. F. VIII specifies that the total dry weight of material taken for micro-Kjeldahl should be in the vicinity of 0.1 Gm. (2), the size of the sample necessary precluded the possibility of using this method because of the obvious difficulty encountered in digestion. It was finally found possible, however, to carry out this digestion using a 500-cc. Kjeldahl flask and from 30 to 50 cc. of sulfuric acid instead of the much smaller quantity limiting the micro procedure. The sample was added in portions at intervals to the acid making the digestion a long and tedious process.

Thus by substituting the regular Kjeldahl method for the micro method it was found possible to carry out determinations with a fair degree of reproducibility.

The results of 20 Kjeldahl determinations are given in Table II.

TABLE II.—NITROGEN DETERMINATION BY KJELDAHL ON 75-MG. ALIQUOTS OF HOMATROPINE METHYLBROMIDE

Mg. Nitrogen Found	% of Theoretical	Mg. Nitrogen Found	% of Theoretical
2.68	94.4	2.71	95.5
2.75	96.9	2.64	93.0
2.91	102.2	2.57	90.5
2.61	91.9	2.77	97.6
2.71	95.5	2.52	89.1
2.67	94.1	2.68	94.4
2.55	89.9	2.82	99.4
2.68	94.4	2.74	96.5
2.95	104.1	2.63	92.0
2.73	96.2	2.69	94.8

DISCUSSION

In comparing the two series of results obtained in Tables I and II, the mean values and standard deviations were determined based on the percentage of theoretical. The latter value was derived by the formula

$$\text{Standard deviation} = \sqrt{\frac{\Sigma d^2}{n}}$$

in which Σd^2 is the sum of the squares of the deviations from the mean and n is the number of determinations. The results of these calculations were found to be

	Mean %	Deviation, %
Reineckate method	96.5	±0.5
Kjeldahl method	95.1	±3.6

Although there is a considerable difference in the size of the samples taken for analysis, 5 mg. in the Reineckate method and 75 mg. in the Kjeldahl method, it is interesting to note that the discrepancy between the means of the two series is not considerable. The large sample required and the low percentage of nitrogen in the Homatropine Methylbromide molecule (3.78%), together with the difficulties encountered in digestion, are probably responsible for the greater deviation in the Kjeldahl method.

The colorimetric Reineckate method, despite the fact that it involves an extraction procedure prior to determination, was found to be simple, rapid, and accurate within 5%. It can be carried out in considerably less time and on a smaller sample than the Kjeldahl determination and has an advantage in that several determinations may be carried out simultaneously with little additional time and labor.

The method is not specific for Homatropine Methylbromide since other nitrogen heterocycles, tertiary amines, and some secondary amines interfere. It is somewhat more specific, however, than the Kjeldahl method.

Since Homatropine Methylbromide is a synthetic alkaloid generally used in tablet form without admixture or with a sedative such as the barbiturates, it is interesting to note that barbiturates do not interfere in the determination since they do not form insoluble Reineckates.

SUMMARY AND CONCLUSIONS

Homatropine Methylbromide can be quantitatively precipitated from aqueous solutions as the insoluble Reineckate by Ammonium Reineckate solution. After isolation by filtration the Reineckate dissolved in acetone can be determined colorimetrically by reference to a standard curve prepared with known quantities of Homatropine Methylbromide.

The compound may be extracted from tablets with water and determinations carried out on the resulting solution.

Comparison of results obtained by the Reineckate method with those obtained by the Kjeldahl method indicate fair agreement of the mean values found and less deviation from the mean with the Reineckate method.

The latter method was found to be simple, rapid, and accurate within 5 per cent in a series of 20 determinations.

It has an advantage in that a number of determinations may be carried out simultaneously and requires a considerably smaller sample than does the Kjeldahl method.

Because of the large sample required for the Kjeldahl, the micro method could not be used. The regular Kjeldahl method with some alteration gave fairly reproducible results.

Although the method is not specific for Homatropine Methylbromide since certain other compounds interfere, it is significant that barbiturates, often used in combination with Homatropine Methylbromide, do not interfere. The method is, however, more specific than the Kjeldahl method.

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The Determination of Arsenic in Organic Compounds*

By C. K. BANKS,† J. A. SULTZABERGER,† F. A. MAURINA,‡ and CLIFF S. HAMILTON‖,§

Techniques of wet combustion of organic compounds and methods of estimating arsenic were studied in an effort to formulate a single procedure which would combine the features of universal applicability, ease of operation, and a high degree of precision and accuracy. Six new variations in procedure were evolved and compared with two standard methods against analytically pure *p*-arsanilic acid. Statistical evaluation of the results of nearly five hundred assays indicated that all six variants were superior to the standard methods examined. Three of the six variants were selected for further examination as to applicability to various types of organic arsenicals. While no one method would suffice for all types, one of the variations was found to be adequate for those types of arsenicals used as drugs. A group of representative official preparations was assayed and the results indicated that the new procedure was applicable as a general method for all official arsenicals. It is recommended that the new procedure be considered for the estimation of arsenic in official preparations.

THE ESTIMATION of arsenic in organic compounds has been the subject of numerous publications. In reviewing these articles the impression is gained that there is little agreement as to the validity of any one method with a given compound and little evidence of the general applicability of many of the methods. The publication of three different methods recently (1–3) is indicative of the general dissatisfaction with existing procedures. A further analysis shows that the precision and accuracy of the methods described have seldom been investigated on a scale sufficiently large so as to ascertain the validity of the claims. Methods have even been recommended in which none of the products tested was of unquestioned composition so as to independently confirm the procedure. Since both research and production control are dependent on these considerations, such an investigation would appear to be warranted.

From a comprehensive point of view methods are needed to determine independently (*a*) the total arsenic present in organic materials, (*b*) total arsenic in biological materials, (*c*) the arsenic present linked directly to carbon in the so-called pentavalent form, (*d*) the arsenic present linked to carbon in trivalent form, and (*e*) inorganic arsenic admixed in organic materials. Some of us have published methods applicable to problems *b* (4) and *d* (5) and an early report on *a* (6). Both *c* and *e* have proved to be difficult of solution. This paper will report further experiences during the past twenty years with the estimation of the total arsenic present in organic materials. During this period approximately twenty thousand research samples and fifty thousand routine control assays have been performed by various procedures. These samples have been representative of nearly every known type of organic arsenical.

The ideal method for arsenic would at one time combine applicability to any type of arsenical, a high order of precision and accuracy (at least five parts per thousand), rapidity of procedure, freedom from manipulative error, and simplicity in operation. Unfortunately, no one method has been found which approaches the ideal in all respects. Methods found to have a high degree of precision and accuracy were frequently not applicable to all types of compounds and those of more general usefulness were frequently either tedious to perform or of a low order of accuracy.

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‡ Analytical Laboratories, Parke, Davis and Co.

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Space will not permit a detailed analysis of the methods tried and a review of the extensive literature on the subject is unnecessary. The problem, from a theoretical point of view, has been considered under three main headings: digestion of organic material, establishment of arsenic in a unique state of valence, and estimation of the arsenic. Under the latter heading, gravimetric procedures were not considered since volumetric methods are sufficiently accurate, are more rapid, and are freer of manipulation and precipitation error. It should be stated that the procedures finally established are not original with us, but rather combinations of the most desirable features of other methods.

Digestion techniques examined are listed in Table I. It is apparent that only four general types are of value: sulfuric acid-sulfates-carbohydrates, permanganate, sulfuric-nitric acids, and sulfuric acid-hydrogen peroxide. While the first mentioned is limited to nonhalogen and organically bound chlorine compounds, because of the extensive experience accumulated by one of us with it (C. S. H.) (7), it was retained with slight modification for reasons that will become apparent.

Consideration of volumetric techniques of estimating arsenic were limited to three: titration of trivalent arsenic with potassium bromate in strong acid, titration of trivalent arsenic with iodine in neutral media, and estimation of pentavalent arsenic by liberation of iodine from potassium iodide in strong acid and determination of the iodine liberated with thiosulfate. Of the three it was apparent that the bromate titration was subject to the least error. Table II shows that the assay of Bureau of Standards arsenic trioxide was quantitative within the manipulative error inherent in the burette used. The titration of arsenic trioxide with iodine is of known value, but the neutralization of the acid present and the inherent variability of iodine solutions (8) make the method undesirable. The determination of arsenic from the pentavalent form by liberation of iodine from iodides is well known. It also has a number of inherent sources of error. The liberation of iodine is greatly

TABLE I

Digestants	Interfering Substances	Comments
1. H_2SO_4	Halogens	Not generally applicable
2. H_2SO_4 + Na_2SO_4 (or K_2SO_4)	Halogens	Valence of arsenic uncertain
3. H_2SO_4 + Na_2SO_4 + carbohydrates	Halogens	Excellent when no halogens present; arsenic trivalent
4. $HClO_4$	.....	Highly variable
5. $HClO_4$ + H_2SO_4	.....	Dangerous
6. $HClO_4$ + HNO_3	.....	Dangerous
7. $HClO_4$, H_2SO_4, HNO_3	.....	Dangerous
8. $(NH_4)_2S_2O_8$ + H_2O	.....	Highly variable
9. $(NH_4)_2S_2O_8$ + H_2SO_4	.....	Highly variable
10. $KMnO_4$ + H_2SO_4	Bromine, iodine	Good
11. H_2SO_4 + HNO_3	Bromine, iodine	Good
12. HNO_3 digestion followed by H_2SO_4	None	Good
13. H_2SO_4, HNO_3 (fuming)	As in 11 and 12	Good
14. H_2SO_4 + KNO_3	Halogens	Good
15. H_2SO_4 (fuming), HNO_3 (fuming)	As in 11 and 12	Good
16. H_2SO_4 + 30% H_2O_2	Halogens	Good
17. 30% H_2O_2 digestion followed by H_2SO_4	None	Good
18. H_2SO_4 (fuming), 30% H_2O_2	As in 16 and 17	Good

TABLE II.—TITRATION OF ALIQUOTS OF ARSENIC TRIOXIDE SOLUTION IN SULFURIC ACID WITH POTASSIUM BROMATE

H_2SO_4 (*N*)	Temp., °C.	No. Determinations	Assay, %
2	70	3	98.2 – 99.3
2	80	5	99.5 ± 0.3
2	90	8	99.7 ± 0.2
4	70	5	99.95 ± 0.08
4	80	5	99.98 ± 0.06
4	90	5	99.99 ± 0.04
6	70	5	99.99 ± 0.05
6	80	5	100.00 ± 0.04
6	90	5	99.98 ± 0.06
8	70	5	99.97 ± 0.05
8	80	5	99.95 ± 0.05
8	90	5	99.90 ± 0.10
10	70	5	99.87 – 100.3

influenced by time, temperature, and acidity as indicated in Table III. The effect of oxygen on iodides in strong acid must also be considered, requiring blanks. The effect of temperature, acidity, time, and the presence or absence of iodine greatly varies the blank (Table IV). In particular, the blank is increased by the presence of iodine. This feature of the blank is generally ignored, resulting in a constant small error since iodine is always present in actual determinations. These many variables, along with the necessity of several standard solutions, make such a determination undesirable for routine assays and not advisable for research assays if better procedures are available. From these considerations, further research was concentrated on procedures using the bromate titration.

TABLE III

(Aliquots containing exactly 0.06154 Gm. of As_2O_3 were oxidized with $KMnO_4$ in H_2SO_4, the excess discharged with H_2O_2, $KMnO_4$ added to a faint pink and discharged with oxalic acid. An excess of KI was added and after varying time intervals, the liberated iodine determined. Sulfuric acid, 25 ml. (1:2) was present in all determinations. Blank determinations were made for each run.)

Dilution, Ml.	Temp., °C.	Time, Min.	Recovery, %
125	10	60	95.2
125	20	60	98.3
125	25	30	97.0
125	25	60	99.2
75	25	60	100.3
100	25	60	99.6
125	25	60	99.2
175	25	60	83.7
200	25	60	72.3

Potassium bromate very closely approximates the ideal standard. It is easily obtained as a pure salt, stable in solution for at least three years and has an easily detectable end point.

Since potassium permanganate digestions limit the determination to the liberation of iodine (9), they were not considered more extensively than necessary to establish the precision of the present U. S. P. XII method for arsphenamine [modified from the Rapp and Lehman method (10)]. The sulfuric acid-sodium sulfate-carbohydrate digestion was modified by a number of simplifications from the procedure originally reported (6).

TABLE IV.—BLANK TITRATIONS OF SOLUTIONS CONTAINING 25 ML. OF SULFURIC ACID (1:25) AND 20 ML. POTASSIUM IODIDE T. S.

Vol., Ml.	Temp., °C.	Time, Min.	Blanks, 0.1 *N* $Na_2S_2O_3$, Ml. No I_2	10 Ml. 0.1 *N* I_2 Added
125	10	60	0.06	0.19
125	20	60	0.09	0.27
125	30	60	0.12	0.37
125	40	60	0.14	0.46
125	25	30	0.06	0.18
125	25	60	0.12	0.25
125	25	90	0.13	0.39
75	25	60	0.13	0.30
100	25	60	0.17	0.31
125	25	60	0.12	0.25
150	25	60	0.06	0.22
175	25	60	0.05	0.19
200	25	60	0.05	0.16

TABLE V.—EFFECT OF TIME ON ARSENIC TRIOXIDE IN SULFURIC ACID AT THE FUMING POINT

(Aliquots of arsenic trioxide (ca. 25 mg.) were digested in 25 ml. of concentrated sulfuric acid with 0.5 Gm. hydrazine sulfate at a temperature sufficient to cause fumes to rise to the necks of 125-ml. Erlenmeyer flasks.)

Time at Reflux, Min.	No. Determinations	Recovery, %
10	4	99.97– 100.06
15	6	99.99 ± 0.04
20	6	99.98 ± 0.05
30	6	99.97 ± 0.08

The volume of acid was increased slightly, the titration volume reduced, the large excess of starch replaced by the cigarette paper used in weighing and the carbon dioxide atmosphere omitted. It was found that arsenic trioxide in hot sulfuric acid was relatively insensitive to the oxidizing action of the acid or air, allowing greater variation in the digestion without introducing error (see Table V). Also, it was noted that bromine and iodine always interfered with the assay, chlorine only when it was present originally as ionic halogen, or else easily liberated halogen. Since the arsenic trihalides are easily volatilized, this can be understood. The unusual behavior of bromine was not understood until it was reported recently that arsenic pentabromide is also volatile (11), explaining the reason all of the methods gave highly variable results when bromide was present. The potassium bromate titration end point was detected electrometrically and internally with methyl orange indicator.

TABLE VI.—FREQUENCY OF ASSAY VALUES FOR *p*-ARSANILIC ACID BY EIGHT METHODS

(Calculated for As = 34.51%)

34.00 + %	Method A	B	C	D	E	F	G	H
<0.04	..	..	..	..	..	..	1	1
0.04–0.08	..	..	..	2	..	..	1	..
0.09–0.13	..	1	1	1	..	..	1	1
0.14–0.18	1	2	1	4	..	..	1	2
0.19–0.23	..	2	2	1	..	1	2	..
0.24–0.28	..	3	3	3	..	1	..	..
0.29–0.33	1	4	5	1	1	1	1	1
0.34–0.38	4	6	5	11	1	1	..	..
0.39–0.43	9	10	10	10	4	3	2	2
0.44–0.48	18	10	11	12	4	2	..	2
0.49–0.53	28	20	24	14	7	6	5	3
0.54–0.58	20	9	12	13	3	2	1	4
0.59–0.63	10	9	10	12	1	3	2	3
0.64–0.68	6	6	4	2	2	1	1	1
0.69–0.73	1	5	3	4	..	2	1	3
0.74–0.78	1	4	3	2	1	1	..	1
0.79–0.83	..	2	2	3	..	..	..	1
0.84–0.88	..	3	1	1	..	..	..	1
0.89–0.93	..	2	1	2	..	..	1	..
0.94–0.98	..	1	1	1	..	..	1	1
>0.98	..	..	..	..	..	..	..	3
Total	99	99	99	99	24	24	21	30

The peroxide-sulfuric acid digestion was found to give excellent results, except that large amounts of halogens interfered. It was then found that prior addition of the peroxide, followed by the acid, gave excellent results, even when bromides were present. Carbohydrates, hydrazine, hydroxylamine, and other agents were generally applicable to reduce the arsenic to the trivalent state. Hydrazine sulfate proved to be as useful as any and far more rapid in action than starch.

The sulfuric-nitric acids method was also examined more closely since it was found that a few compounds having ring arsenic were not decomposed by the other methods. It was found that a predigestion with fuming nitric acid removed the possibility of halogen interference. The same reducing agents were tried as before but the results were strikingly different.

Highly purified *p*-arsanilic acid was used

TABLE VII.—STATISTICAL DATA FOR ALL ASSAYS CONSIDERED AS ONE GROUP[a]

Method	No. of Assays in Each Group	No. of Groups	Av., % As (Theory 34.51)	Standard Deviation	Standard Error	Precision, Absolute, %	Precision, Parts per 1000	Error, Deviation from Theory	Accuracy, Parts per 1000
A	3	33	34.51	0.09	0.009	0.02	0.6	0.001	0.6
B	3	33	34.52	0.17	0.017	0.05	1.4	0.007	1.7
C	3	33	34.52	0.13	0.013	0.04	1.1	0.008	1.4
D	3	33	34.50	0.18	0.018	0.05	1.4	0.010	1.7
E	3	8	34.50	0.10	0.020	0.06	1.7	0.012	2.0
F	3	8	34.50	0.14	0.028	0.08	2.3	0.012	2.6
G	3	7	34.43	0.29	0.063	0.18	5.0	0.07	7.0
H	6	5	34.68	0.35	0.064	0.18	5.0	0.17	10.0

[a] See footnote *b* (Notes) under Table VIIa.

TABLE VII*a*—STATISTICAL DATA FOR ASSAYS AS GROUPS[a, b]

Method	α	β	Δ	Standard Deviation of Group Mean	Precision of Group Mean	Predicted Replicability Expected Precision of the Mean When the Assays in Group Number: 2, Absolute, %	2, Parts per 1000	3, Absolute, %	3, Parts per 1000	Time Required for 3 Assays
A	0.5108	0.2068	2.46	0.046	±0.02	±0.18	5	±0.12	3	60
B	1.78	1.29	1.36	0.11	±0.05	±0.34	10	±0.29	8	50
C	1.14	0.52	2.20	0.07	±0.04	±0.24	7	±0.21	6	40
D	1.78	1.30	1.37	0.12	±0.05	±0.35	10	±0.30	8	30
E	0.06	0.17	0.33	0.09	±0.09	±0.28	8	±0.25	7	240
F	0.11	0.34	0.28	0.13	±0.12	±0.38	11	±0.36	10	180
G	1.23	0.46	2.64	0.16	±0.17	±0.83	24	±0.64	19	80
H	1.69	1.84	0.74	0.28	±0.35	±0.95	28	±0.90	26	50

[a] The number of assays in each group and the number of groups upon which these data are based are indicated in the second and third columns of Table VII.

[b] NOTES:

1. All experimental limits calculated for P = 0.01 from "Student's *t*" tables.
2. All predicted limits calculated with $t = 3$ or the P = 0.01 value from "*t*" tables, whichever was larger.
3. $\alpha = \Sigma X^2 - \Sigma M^2/m$, $\beta = \Sigma M^2/m - mn\bar{X}^2$, $\Delta = \frac{\alpha}{\beta}\left(\frac{m-1}{m}\right)$, where X = observed values, $\bar{X}$ = mean, M = group means, m = number of groups, n = number of replicates in group.
4. Predicted precision for three assays $Z = \pm t\sqrt{\frac{\beta}{n(m-1)}}$; for two assays $Z' = \pm t\sqrt{\frac{\beta}{n(m-1)}\left(1+\frac{\Delta}{2m}\right)}$

as a standard arsenical. It may be prepared in better than 99.97 per cent purity, verifiable by independent procedures. It was selected in preference to any of the official arsenical preparations as none of these products was available in quantity in such a degree of purity.

Eight procedures were investigated including the Association of Official Agricultural Chemists method, as representative of titration with iodine (Experimental—method G), and the U. S. P. XII arsphenamine procedure (method H), as representative of permanganate digestions. Six variations developed in our laboratories completed the studies (methods A to F, inclusive). Doctorate students, analysts, analytical chemists, and research chemists have used these methods. The values obtained were recorded in sequence with no selection of data. Under methods A to D, inclusive, only the last ninety-nine determinations were recorded. The assays were generally performed in groups of three. Many individuals have used four of the methods and several have used all of the methods. Table VI shows the frequency distribution of four hundred and ninety-five such assays. The frequency distributions for 0.05% intervals (Table VI) were also converted to percentile frequencies and the resulting values plotted in the accompanying 4 graphs (Figs 1–4). The histograms thus formed were adjusted to continuous frequency curves which are indicated in the figures. The statistical significance of the deviations from normal distribution indicated by the curves is considered in the Discussion. The individual assay values and the group means were analyzed by standard methods of statistical analysis and are reported in condensed form in Tables VII and VII*a*. From the deviations so calculated, the probable precisions and accuracies were also calculated. Table VIII lists a number of representative type compounds analyzed by several methods. It is of interest that no reagent blank was used with methods A, C, E, and F and only the indicator blank with B and D.

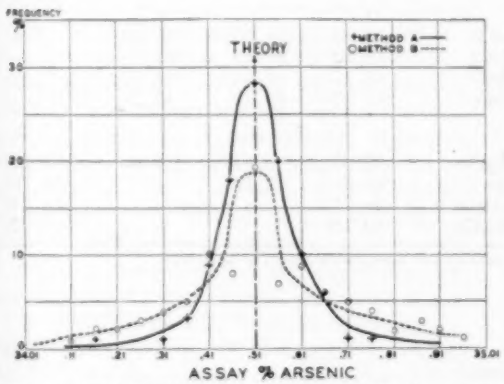

Fig. 1.—Distribution of assays, methods A and B.

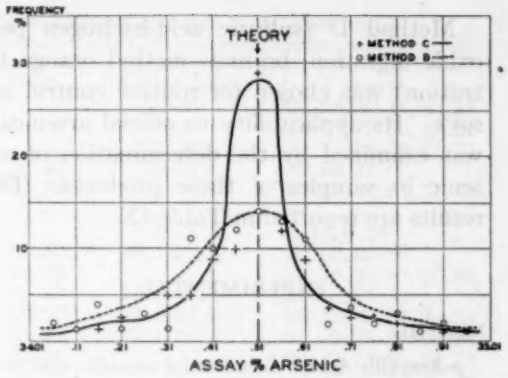

Fig. 2.—Distribution of assays, methods C and D.

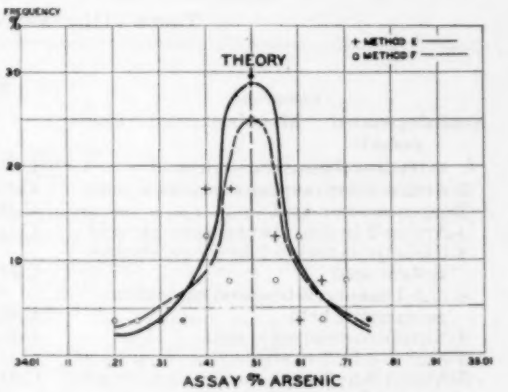

Fig. 3.—Distribution of assays, methods E and F.

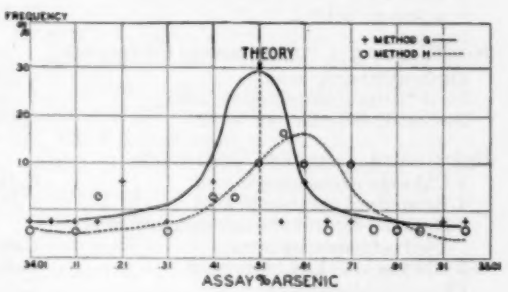

Fig. 4.—Distribution of assays, methods G and H.

Method D (sulfuric acid-hydrogen peroxide digestion, bromate-methyl orange titration) was chosen for routine control assays. Its applicability to official arsenicals was examined by the determination of arsenic in samples of these products. The results are reported in Table IX.

EXPERIMENTAL

Materials

***p*-Arsanilic Acid.**—Commercial arsanilic acid containing less than 0.1% *ortho* isomer was dissolved in boiling water, treated with charcoal (Norite), filtered and the crop of crystals separating at room temperature removed. These crystals were then recrystallized twice by dissolving in a minimum of boiling water, diluting with an equal volume of hot water, and filtering by suction through an ultrafine sintered glass funnel. The hot solution from the last filtration was chilled rapidly with agitation to obtain fine crystals. The crystals were centrifuged, the liquid decanted, and the product washed twice with distilled water. The crystals were sucked dry in a sintered glass funnel and dried over phosphorus pentoxide at 1 mm. The *p*-arsanilic acid was assayed from diazotizable amine with standard sodium nitrite, using silver and silver chloride electrodes to determine the end point. The samples showed an indicated purity of 100.00 ± 0.05%.

TABLE VIII.—ASSAYS OF TYPICAL ARSENICALS

Compound	Molecular Formula	Per Cent Arsenic			
		Calcd.	Found		
			Method A	Method D	Method E
Nonhalogenated, Monobenzenoid Compounds					
4-Hydroxybenzenearsonic acid	$C_6H_7AsO_4$	34.36	34.42	34.48	34.60
3-Amino-4-hydroxybenzenearsonic acid	$C_6H_8AsNO_4$	32.14	32.04	32.19	....
Benzenearsonic acid	$C_6H_7AsO_3$	37.08	37.14	36.89	36.90
4-Amino-2-hydroxybenzenearsonic acid	$C_6H_8AsNO_4$	32.14	32.16	32.30	....
4-Carbethoxyamino-2-hydroxybenzenearsonic acid	$C_9H_{12}AsNO_6$	24.55	24.55	24.68	....
4-(2,4-Diamino-6-triazinyl)aminobenzenearsonic acid	$C_9H_{11}AsN_6O_3$	22.97	23.00	23.12	....
4-Nitrobenzenearsonic acid	$C_6H_6AsNO_5$	30.32	30.22	30.46	30.54
3-Amino-4-hydroxybenzenearsonous acid[b]	$C_6H_8AsNO_3$	34.51	34.51	34.49	....
5-Amino-2-hydroxybenzenearsonous acid[c]	$C_6H_8AsNO_3$	34.51	34.48	....	....
4-(2,4-Diamino-6-triazinyl)ammoniumbenzenearsonite[d]	$C_9H_{13}AsN_6O_3$	22.84	22.85	22.76[g]	....
4-Arsenosophenylurea[e]	$C_7H_7AsN_2O_2$	33.16	33.23	33.28	....
3-Amino-4-β-hydroxyethoxybenzenearsonous acid[f]	$C_8H_{12}AsNO_4$	27.22	27.26	27.10	....
Nonhalogenated, Polybenzenoid Compounds					
Diphenylarsinic acid	$C_{12}H_{11}AsO_2$	28.58	28.50[h]	*26.68*	28.71
bis-3-Nitrodiphenylarsinic acid	$C_{12}H_9AsN_2O_6$	21.27	21.36[h]	*19.21*	21.10
2-Nitrodiphenylarsinic acid	$C_{12}H_{10}AsNO_4$	24.39	24.52[h]	....	24.31
Halogenated, Benzenoid Compounds					
4-Chlorobenzenearsonic acid	$C_6H_6AsClO_3$	31.68	31.71	31.51	31.49
4-Bromobenzenearsonic acid	$C_6H_6AsBrO_3$	26.66	*22.16*	26.54	26.72
3-Amino-4-hydroxybenzenearsonic acid hydrochloride hydrate	$C_6H_{11}AsClNO_5$	29.72	*25.24*	29.69	....
2-Chlorophenyl dichlorarsine	$C_6H_4AsCl_3$	29.11	....	29.24	28.98
Phenyldichloroarsine	$C_6H_5AsCl_2$	33.60	....	33.45	33.78
Aliphatic Arsenicals					
Ethylarsonic acid	$C_2H_7AsO_3$	48.65	48.60	48.52	....
Ethyldichloroarsine	$C_2H_5AsCl_2$	42.83	*42.45*	42.60	....
Butylarsonic acid	$C_4H_{11}AsO_3$	41.15	41.12	41.32	....
Allylarsonic acid	$C_3H_7AsO_3$	45.13	45.19	45.27	....
Arsenic as a ring element					
Cyclopentamethylenearsinic acid	$C_5H_{11}AsO_2$	42.07	*2.68*[i]	*0.45*[i]	41.87[i]
Methylarsepidine	$C_6H_{13}As$	46.80	*1.27*[i]	....	46.70[i]
Arsanthrenic acid	$C_{12}H_{10}As_2O_4$	40.71	....	....	40.64[i]
9,10-Dichloro-9,10-dihydroarsanthrene	$C_{12}H_8As_2Cl_2$	40.18	....	....	40.02[i]

[a] Average of two assays, assays in italics considered to be outside the acceptable limits of accuracy.
[b] Trivalent organic arsenic, 34.50%.
[c] Trivalent organic arsenic, 34.49%.
[d] Trivalent organic arsenic, 22.88%.
[e] Trivalent organic arsenic, 33.18%.
[f] Trivalent organic arsenic, 27.25%.
[g] Obtained after a triple digestion.
[h] Two-hour digestion followed by carbohydrate reduction used.
[i] After twenty-four-hour digestions.
[j] Digestion prolonged to two hours.

TABLE IX.—ANALYSIS OF OFFICIAL ARSENICAL PREPARATIONS AND RELATED COMPOUNDS

Compound	Empirical Formula	Arsenic, %—Method D[a]		
		Calcd.	Official Limits[b]	Found
Arsphenamine[c]	.....	...	30–32	30.25, 30.11, 30.03
Neoarsphenamine[c]	.....	...	>19	19.52, 19.60, 19.51
Sulfarsphenamine[d]	.....	...	>19	21.17, 21.36, 21.33
Oxophenarsine Hydrochloride[e]	$C_8H_7AsClNO_2$	31.81	30–32	30.98, 31.07
Oxophenarsine Hydrochloride[f]	$C_8H_7AsClNO_2$	31.81	.....	31.82, 31.81, 31.86
Oxophenarsine Hydrochloride Mixture[e]	.....	...	6.0– 6.4	6.18, 6.20
Dichlorophenarsine Hydrochloride[e]	$C_8H_7AsCl_2NO$	25.80	25.3–27.0	25.52, 25.56
Dichlorophenarsine Hydrochloride[f]	$C_8H_7AsCl_2NO$	25.80	.....	25.79, 25.86, 25.89
Acetarsone[f]	$C_8H_{10}AsNO_5$	27.23	27.1–27.4	27.29, 27.32
Carbarsone[f]	$C_7H_9AsN_2O_4$	28.80	26.1–28.8	28.82, 28.87, 28.94
Tryparsamide (Anhyd.)[e]	$C_8H_{10}AsN_2NaO_4$	25.30	25.1–25.5	25.22, 25.27, 25.24
Tryparsamide ($\frac{1}{2}H_2O$.)[e]		24.55	.....	24.75, 24.68, 24.75
Sodium Arsanilate (Anhyd.)[f]	$C_6H_5AsNNaO_3$	31.34	.....	31.46, 31.52, 31.49
Sodium Cacodylate (Anhyd.)[f]	$C_2H_6AsNaO_2$	46.83	45.54–46.95[g]	46.92, 46.87

[a] Corrected for indicator blank. [b] U. S. P. XII, N. F. VII, or N. N. R. [c] Merck. [d] Squibb. [e] Parke, Davis & Co. [f] Research Laboratories, Parke, Davis & Co. [g] Interpolated from U. S. P. XII limits for trihydrate.

Standard Solutions.—All standard solutions were prepared according to Kolthoff and Sandell (12).

Official Arsenicals.—Official arsenicals of indefinite structure were obtained as commercial samples. Those of definite structure were synthesized and purified by published methods. In some instances commercial samples of this type of product were also assayed.

Other Arsenical Products.—The compounds listed in Table VIII were prepared as part of our arsenical research programs.

Method A.—Samples of *p*-arsanilic acid (0.18–0.2 Gm.) were weighed accurately to four places on a cigarette paper. The paper was folded over the sample and dropped into a 300-ml. Kjeldahl flask, 25 Gm. anhydrous (reagent grade) sodium sulfate added and then 30 ml. of concentrated sulfuric acid. The samples were digested over a low flame until charred and the foaming had ceased and then over a high flame until a very pale amber or barely colorless. If the digest became colorless for any period of time due to an oversight, another cigarette paper was added and the digestion repeated. After cooling, 75–100 ml. of water was added cautiously and the contents transferred to a 600-ml. beaker, diluted to 400 ml., an additional 25 ml. of concentrated sulfuric acid added and titrated while hot with 0.05000 *N* potassium bromate solution, using a platinum and calomel electrode system attached to a voltage measuring instrument (potentiometer, titrimeter, capillary electrometer, vacuum tube null-point instrument, etc.). The electrode potential changed less than 0.005 volt until the end point was approached. Within a few tenths of a milliliter of the end point, a temporary voltage fluctuation was noted, followed by a permanent deflection of 0.2–0.3 volt at the end point. No blank was used.

Samples of exactly 0.1873 Gm. may be read directly from the burette in per cent when exactly 0.05 *N* bromate is used.

Method B.—The sample was digested as in A except that a 250-ml. Erlenmeyer flask was used. When the digestion was completed, 100 ml. of water was added and the titration carried out while hot using 2 drops of methyl orange indicator. A correction for the methyl orange blank was made.

Method C.—Weighed samples (0.18–0.2 Gm.) were placed in 250-ml. Erlenmeyer flasks and 15 ml. of 30% hydrogen peroxide added, and then slowly with shaking 25 ml. of concentrated sulfuric acid. The digestion was carried out on a hot plate set at such a temperature as to cause the condensation line to approach to within $\frac{1}{2}$ inch of the top of the flask. If the solution did not turn colorless, the digests were cooled slightly and peroxide added dropwise until the solution cleared. The digestion was continued for ten minutes after clearing and then cooled; 0.2 Gm. hydrazine sulfate added through a long-necked powder funnel and the digests either boiled vigorously over a flame for fifteen minutes or allowed to heat on the hot plate for thirty minutes. After cooling, 60 ml. of water was added, the contents transferred to a 600-ml. beaker, diluted to 400 ml., 35 ml. of sulfuric acid added and titrated hot as in A.

Method D.—The digestion was carried out as in C and, after the addition of 60 ml. water to the digest, the samples were titrated as in B.

Method E.—Samples were weighed out as in A, 5 ml. of fuming nitric acid (sp. gr. 1.50) added, heated to boiling, and then 25 Gm. of anhydrous reagent grade sodium sulfate and 30 ml. of sulfuric acid added. The digestion was continued for thirty minutes, then an additional 5 ml. of fuming nitric acid added cautiously and the digestion repeated. Three cigarette papers, or a 5-cm. sheet of ashless filter paper, were added and the digestion and titration completed as in A.

Method F.—The initial digestion was the same as E. Instead of adding paper, hydrazine sulfate

was added and the remainder of the procedure duplicated C.

Method G.—Association of Official Agricultural Chemists' method.

Method H.—Arsphenamine procedure, U. S. P. XII.

DISCUSSION OF RESULTS

The composite results as collected in Table VI qualitatively suggest that all of the assay methods are measuring the same theoretical value. The one possible exception would be method H. In order to evaluate the data more accurately, statistical treatment was applied as indicated in Tables VII and VII*a*. Each series of values appeared to be homogeneous within itself. Method H, although giving a chi-square value of P = 0.17 could not be considered as improbable. The data in their entirety were nonhomogeneous, as were combinations of any two methods except B and D. These two methods would appear to have a common random error and since the similarity was limited to the same end-point indicator, it suggested that the digestion methods for A, B, C, and D were equivalent within the precision of the methyl orange end point. Comparison of the methods indicated that the theoretical value of *p*-arsanilic acid was within the precision of all of the methods, method H again being a borderline case. Based on standard error, method A was the most precise, followed by C, B, D, E, F, G, and H in that order. The accuracy as compared to theory showed the first five methods as being of a high precision, F less so, with G and H far less accurate.

A consideration of the data as a whole does not predict the degree of replicability of the methods. The data were also treated as individual groups as they originally occurred according to the method reported by Mandel (13) so as to introduce the space-time factor. The notations, α, β, and Δ are proportional to his A, B, and D.

The expected precision clearly indicates that method A is by far the most precise followed by C, E, B, D, F, G, and H in that order. The numerical values indicate that with three replicates, method A is duplicable within $\pm 0.12\%$ or three parts per thousand, while for B, C, D, and E the indicated per thousand precision is less than $\pm 0.3\%$ or eight parts per thousand. For two replicates the indicated precisions are $\pm 0.18\%$ and $\pm 0.35\%$. Method F showed slightly greater variance while methods G and H can hardly be considered as being adequately precise for any truly quantitative procedure. Methods G and H would show greater precision if known compounds were used as blanks.

Careful consideration led to the conclusion that the methods of choice for our purposes were A, D, and E. For research purposes, if halogen is absent, method A is outstandingly superior. For halogen compounds and for routine analyses, method D is adequately accurate and precise, very rapid, and easier to perform. Method C can be chosen in preference to D if potentiometric equipment is available. Method B has nothing to recommend its use over C or D, indicating that if the methyl orange end point is used, no advantage is gained by using the Cislak–Hamilton digestion method. One or two exceptions to this statement have been noted where the higher digestion temperature of A and B proved to be advantageous. Method E is clearly to be preferred to F. The nitrogen oxides present during the digestion are apparently not removed as quantitatively by hydrazine as by carbohydrates.

Method D proved adequate for nearly all compounds. Compounds containing heterocyclic rings were assayed more accurately by methods A and B than by D. However, by repeating the digestion with hydrogen peroxide, method D yielded values which were also acceptable. Compounds containing the arsinic acid group or arsenic in a heterocyclic ring were not decomposed by method D. Arsinic acids assayed by method A when halogen was absent but ring arsenic was made available for analysis only by method E.

The assay of official arsenicals by method D indicates that the expected precision and accuracy were attained. This contrasts with the experience of Jorgensen (2) who found the very similar U. S. P. XII dichlorophenarsine hydrochloride method to be variable as to results and blank. It has been our experience that such variation is due to incomplete destruction of excess hydrazine and that no blank, other than the indicator blank, is found when the hydrazine is properly destroyed.

RECOMMENDATION

It is recommended that the revision committees of the United States Pharmacopœia and the National Formulary seriously consider the described method D as a general procedure for all official arsenicals.

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A Method for Determining the Size and Relative Weight of Penicillin Particles and Its Importance in Preparing Adequate Preparations of Liquid Penicillin in Oil and Wax*,†

By VELMA L. CHANDLER, MONROE J. ROMANSKY, HENRY WELCH, JAY A. ROBINSON, WILLIAM W. ZELLER, HARRY F. DOWLING, and HAROLD L. HIRSH‡

The efficiency of penicillin in oil and wax is influenced by the size and relative weight of penicillin particles. A method is described by which sizes and relative weights of penicillin particles may be determined. The results by the method described are correlated with blood levels obtained following injections of penicillin in oil and wax.

THE difficulties inherent in the administration of the original viscous penicillin in oil and wax appeared to have been overcome when a method for making the mixture fluid at room temperature was developed. During recent months, however, studies by Hirsch and his associates (1) and Romansky (2) seemed to indicate that the liquid preparations of penicillin in oil and wax were not as efficient as the original solid preparations in prolonging blood concentrations of this drug. Consequently, a comprehensive study was undertaken to determine the relative merits of these two types of preparations. A total of 252 patients were treated with either the liquid or solid material obtained from eleven manufacturers. Each patient was given a single intramuscular injection of 300,000 units of penicillin in oil and wax contained in 1 cc. and blood for penicillin assay was collected at the twelfth, sixteenth, twentieth, and twenty-fourth hour after administration. The detailed results are published elsewhere (3).

During the course of this study it became apparent that some factor other than the physical state and type of penicillin (amorphous or crystalline) determined the efficiency of the penicillin in oil and wax preparations. After a review of the variables, it appeared that the size (relative weight) of the penicillin particles was the most important single factor which influenced prolongation of blood concentrations of penicillin. It therefore became important to devise a method for determining size of penicillin particles in oil and wax preparations. After study of many individual lots of penicillin in oil and wax by various techniques the following procedure was adopted.

EXPERIMENTAL

Method.—After the vial containing the fluid preparation of penicillin in oil and wax is shaken vigorously and inverted several times to insure an even suspension of the penicillin particles, a sample is immediately withdrawn into a dry tuberculin syringe (using a dry needle of 18 gauge). Several drops are rejected from the needle and approximately one minim of the mixture is placed in a test tube containing 3 ml. of light mineral oil. The contents of the stoppered tube are gently mixed for at least one minute until an even suspension is obtained. A bacteriological loopful (2 mm. inside diameter, size 22 wire) of the suspension is immediately placed on each ruled chamber of a bright line hemocytometer. Preliminary examination with the low power objective of the microscope is made to confirm the even distribution of particles in both chambers. It is not necessary to use a cover slip. To measure the size of the penicillin particles, a magnification of 430 or 440 diameters and a calibrated ocular micrometer are employed. Two types of penicillin particles are usually encountered: plate crystals (usually crystalline sodium) or particles of amorphous calcium and rod-shaped crystals (usually crystalline potassium). Since the length and width of the plate particles are approximately the same, only the length of one side is measured; while in the case of the rod-shaped crystals, the width as well as the length is determined. The thickness is assigned a value of one since this dimension is assumed to be approximately the same for all. In making the count, a large particle is centered in the microscopic field

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and its dimensions determined. Subsequently all particles in this field are measured. A second microscopic field containing a large particle is similarly examined and this process is continued until at least 200 particles have been measured. In view of the fact that particles under 5 microns in length were found to be relatively insignificant in terms of weight, they are disregarded.

To determine the relative weight of the plate penicillin particles in oil and wax mixtures the particles are grouped according to length as follows: 5 to 14 microns, 15 to 29, 30 to 49, 50 to 69, 70 to 99, 100 to 149, 150 to 199, 200 to 249, 250 to 299, and 300 to 350. The per cent of the total particles according to length is then calculated for each group. The midpoint of the lengths for each group is then determined and that of the smallest group (5 to 14 microns) or 9.5 microns is arbitrarily assigned a value of one. The ratio of the midpoint of each group to the smallest group is calculated. Thus the particles in the 15 to 29 microns group, with a midpoint of 22 microns, are 2.3 times as long as those in the 5 to 14 microns group. The ratio of the other groups to the smallest are shown in Table I.

To determine the area of the plate particles, the figure representing the ratio of midpoints is squared. Since the thickness and the density of the penicillin particles are essentially the same, the ratio of midpoints squared multiplied by the per cent of total particles in each group yields the relative weight for that group. The per cent of the total relative weight for the individual group is then calculated. For the rod-shaped crystals, the computations are similar except that the midpoint of each group is multiplied by the average width of the particles in that group as shown in Table II. It is appreciated that the assumption that all particles are of approximately the same thickness and density, that the midpoints are an accurate measure of the average length of the particles, and that the choice of microscopic fields for examination by the analyst is purely subjective, may result in relatively large errors. However, in spite of these possible errors in the method, it has, in our hands, correlated well with the clinical studies. In addition, a close correlation of results in particle size measurement of a given sample of penicillin in oil and wax has been obtained by several experienced technicians.

After the method for determining the size of the penicillin particles was established and the formula for the determination of the relative weight of the particles was devised, all of the individual lots of penicillin in oil and wax used in this study were examined and the relative weight of the penicillin particles calculated. In order to determine the influence of the size and relative weight of the penicillin particles on the prolongation of the blood level these calculations were correlated with the blood

TABLE I.—METHOD FOR DETERMINING RELATIVE WEIGHT OF PLATE PARTICLES OF PENICILLIN

Group According to Length of Particle, Microns	Midpoint	Ratio of Midpoints	Ratio² (Length × Width)	×	% of Total Particles Determined by Length	=	Relative Weight of Group	% of Total Relative Weight	
5–14	9.5	1	1.0	×	40.0	=	40.00	1.77	
15–29	22.0	2.31	5.34	×	27.0	=	144.18	6.39	
30–49	39.5	4.16	17.31	×	19.0	=	328.89	14.57	
50–69	59.5	6.26	39.19	×	6.0	=	235.14	10.42	
70–99	84.5	8.89	79.03	×	3.0	=	237.09	10.50	77.27
100–149	124.5	13.10	171.61	×	2.5	=	429.03	19.01	
150–199	174.5	18.36	337.09	×	2.5	=	842.73	37.34	
200–249	224.5	23.63	558.38	×	0		.....	.....	
250–299	274.5	28.89	834.63	×	0		.....	.....	
				Total relative weight		=	2257.06		

TABLE II.—METHOD OF DETERMINING RELATIVE WEIGHT OF ROD-SHAPED PARTICLES OF PENICILLIN

Group According to Length of Particle, Microns	Midpoint	Ratio of Midpoints	×	Av. Width, Microns	×	% of Total Particles Determined by Length	=	Relative Weight of Group	% of Total Relative Weight	
5–14	9.5	1.0	×	4.7	×	23	=	108.10	1.64	
15–29	22.0	2.31	×	7.06	×	20	=	326.17	4.96	
30–49	39.5	4.16	×	8.89	×	21.5	=	795.12	12.09	
50–69	59.5	6.26	×	11.96	×	13	=	973.30	14.80	
70–99	84.5	8.89	×	11.33	×	10	=	1007.24	15.32	
100–149	124.5	13.10	×	14.60	×	5.5	=	1051.93	16.00	81.30
150–199	174.5	18.36	×	16.40	×	5	=	1505.52	22.89	
200–249	224.5	23.63	×	15.4	×	1	=	363.90	5.53	
250–299	275.0	28.95	×	15.4	×	1	=	444.91	6.76	
					Total relative weight		=	6576.19		

levels obtained for each of the lots. As a result of this correlation it was found that penicillin in oil and wax preparations could be classified as the "large particle type" when 50% of the total relative weight was made up of particles of 50 microns or more in length, and as the "small particle type" when the particles under 50 microns in length constituted more than 50% of the total relative weight. This correlation is shown in Table III.

TABLE III.—PERCENTAGE OF PATIENTS WITH MEASURABLE BLOOD CONCENTRATIONS[a] FOLLOWING A SINGLE INTRAMUSCULAR INJECTION OF VARIOUS TYPES OF PENICILLIN IN OIL AND WAX
(300,000 units in 1 cc. of oil and wax)

Type of Penicillin	No. of Patients	12 Hr.	16 Hr.	20 Hr.	24 Hr.
Large Particle					
Solid					
Crystalline	20	100	100	88	90
Amorphous	20	100	100	90	80
Liquid					
Crystalline	37	100	100	100	92
Amorphous	34	97	94	85	82
Small Particle					
Solid					
Crystalline	19	100	100	94	90
Amorphous	48	100	100	83	71
Liquid					
Crystalline	24	100	91	83	56
Amorphous	50	96	75	65	41

[a] *B. subtilis* serial dilution method (4, 5).

It is obvious that with the solid preparations of penicillin in oil and wax, on the basis of the incidence of detectable blood levels at the four intervals, the size and relative weight were of little or no importance as far as the prolongation of blood levels was concerned. With the liquid preparations, however, it can be seen that the size and relative weight of the particles influenced the absorption from the intramuscular site and consequently the duration of penicillin in the blood. Apparently the larger particle material is absorbed more slowly and the levels in the blood consequently prolonged. Liquid preparations containing at least 50% of the total relative weight as large particles gave levels which were comparable to the solid preparations. The small particle size penicillin in oil and wax of the liquid type gave levels which were definitely inferior to the "solid" and "large particle" types of preparations.

DISCUSSION

As a result of this study it can be concluded that at present the optimal preparation of penicillin in oil and wax (containing 300,000 units of crystalline penicillin in 1 cc. of a mixture of oil with 4.8% beeswax) will give levels for twenty-four hours in 90% to 92% of patients. Furthermore, several factors influenced the efficiency of this preparation. All other things being equal, crystalline penicillin in oil and wax was superior to the amorphous type. With the solid preparations, the prolongation of blood concentrations of penicillin were, in general, the same, regardless of the size of the penicillin particles. The results with the liquid preparations were as good as those with the solid when the penicillin particles were large size. Liquid preparations containing penicillin particles of small size gave definitely inferior results. Considering the ease of administration, the purity of the penicillin, and the duration of this antibiotic in the blood, the liquid preparations of crystalline penicillin of large particle size are recommended as the most satisfactory form of penicillin in oil and wax.

SUMMARY

1. The size and relative weight of penicillin particles influence the efficiency of penicillin in oil and wax in a liquid preparation.

2. A method for determining the size and relative weight of the penicillin particle is presented.

3. Penicillin in oil and wax is considered to be the "large particle type" when 50 per cent of the total relative weight is made up of particles of 50 microns or more in length, and "small particle type" when the particles under 50 microns in length constituted more than 50 per cent of the total relative weight.

4. The correlation of the results of these determinations with the blood levels obtained following 1-cc. injections of the preparations is demonstrated.

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The Assay of Solanaceous Drugs with the Aid of an Adsorption Column*,†

By NAOMI G. BROWN, ERNST R. KIRCH, and GEORGE L. WEBSTER

A procedure for the assay of solanceous drugs and some of their preparations is described which makes use of the principle of selective adsorption. Compared with the official method, this procedure gives more precise and accurate results and requires less time.

ADSORPTION procedures as a means of isolating plant constituents have been known since Tswett (1) first used them in the isolation of plant pigments in 1906. The basic technique as described by him was used and adapted by many workers for the purpose of qualitative separation of plant constituents. Little work was reported in the literature on the use of the procedure for quantitative analysis of drugs until the last decade.

In 1937 Valentin (2) applied adsorption procedures to the assay of pharmaceutical preparations, including Tincture of Digitalis. Kondo (3) during the same year was able to separate morphine and thebaine, and in 1940 the chromatographic separation of morphine, codeine, narcotine, and papaverine was reported by Levi and Castelli (4).

Valentin and Franck (5) developed procedures for the determination of the alkaloids in Tincture of Belladonna, Infusion of Coffee, Extract and Fluidextract of Cinchona, and Tincture of Strychnine. La-Rocca and Burlage (6), in 1945, were able to effect a separation of strychnine and brucine from known mixtures and from the preparations of Nux Vomica. In 1946 Mühlemann and Tobler developed chromatographic-titrimetric methods for the determination of the alkaloids in Kola seeds and their galenical preparations (7) and for Colchicum seeds and Tincture of Colchicum (8).

The official assay for the alkaloid content of the solanaceous drugs was introduced in its present form into the U. S. P. Supplement of 1939. This method requires approximately twenty hours, and once the extract is obtained the constant attention of the operator is needed until the assay is completed. In this paper we are reporting the application of selective adsorption to the assay of belladonna, hyoscyamus, and stramonium.

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EXPERIMENTAL

The work of Merz and Franck (9) has shown that activated alumina is a satisfactory adsorbent for the removal of the alkaloids of belladonna, hyoscyamus, and stramonium from the ether extracts of these drugs. Experiments using ion-exchange resins, Lloyd's Reagent, Magnesium Oxide, Alumina, and other adsorbents confirmed the earlier work that alumina was the most suitable for the purposes of this investigation. It is easy to handle, can be reactivated by heating in an oven, and is readily obtainable.

Ethyl ether was found to be suitable both for the extraction of the alkaloids from the plant material and for the adsorption of these on the column. The use of ether also fits in well with the official procedure in which it is used to extract the crude drug.

Reagents.—

Activated Alumina, Grade F-20, Mesh, Minus 80
Aluminum Ore Co., East St. Louis, Ill.
Ethyl Alcohol, U. S. P.
Stronger Ammonia T. S., U. S. P.
0.02 *N* Sulfuric Acid (standardized solution)
0.02 *N* Sodium Hydroxide (standardized solution)
Valser's Reagent, U. S. P.
Ethyl Ether, U. S. P.

Adsorption Column.—A piece of glass wool is fitted into the bottom of a tube 300 mm. in height and 15 mm. in diameter. Activated alumina is then packed into the glass tube to a height of 200 mm. The column is set into a filtering tube with a beaker below it. When packed properly the column can be used without suction.

Procedure for Crude Drugs.—Ten grams of powdered belladonna leaves is placed into an extraction thimble, and the latter is inserted into a Soxhlet extractor. The drug is moistened with 10 ml. of Stronger Ammonia T. S. and 20 ml. of ether and mixed thoroughly. The mixture is macer-

ated for one hour, then extracted for three hours, using ether as the solvent. The extract is adjusted to 100 ml., and a 10-ml. aliquot of the extract is transferred with a pipette to the column. The adsorbed alkaloids are eluted with ethanol. The first eluate is yellow in color and consists principally of an ether-alcohol solution of carotenes. The appearance of alkaloid in the eluate coincides with the elution of chlorophylls from the column. At this point the receiver is changed and the green-colored eluate is collected. About 25 to 40 ml. of alcohol is needed for complete elution of the alkaloids, but the eluate is tested to make sure that desorption is complete. This is determined by placing a few drops of the eluate on a watch glass, evaporating the alcohol, dissolving the residue in 0.5 *N* sulfuric acid, and then adding a drop of Valser's Reagent. (Another good indication that elution is complete is the disappearance of the green color of the chlorophyll band on the column.)

After elution is complete, the solution is evaporated to dryness on a water bath, and the heating is continued for fifteen minutes. The residue is dissolved in a small portion of ether, 15 ml. of 0.02 *N* sulfuric acid is added, and the ether is removed by evaporation. If the color of the chlorophyll interferes in the titration, the acid solution is transferred to a clean, dry flask or beaker. This interference is usually avoided by allowing the ether to evaporate, and then swirling the beaker so that the chlorophyll adheres to the sides, leaving a clear solution. The excess acid is titrated with 0.02 *N* sodium hydroxide, using methyl red as the indicator.

This same procedure is used for the assay of stramonium, hyoscyamus (using 20 Gm. of powdered leaves), and belladonna root. Since no chlorophyll is present in the root, testing the eluate with Valser's Reagent is essential.

Procedures for the Preparations.—Powdered Extract of Belladonna and Pilular Extract of Stramonium: Three to four grams of the extract is placed in an extraction thimble and mixed with an equal bulk of acid-washed sand. The maceration and extraction are carried out in the same way as directed for the crude drug, and a 20-ml. portion of the ethereal extract is used for adsorption.

TABLE I.—RESULTS OBTAINED BY VARYING THE TIME OF MACERATION AND THE VOLUME OF AMMONIUM HYDROXIDE[a]

Time, Hr.	Solvent	Alkaloid, %
Overnight (16)	U. S. P.[b]	0.747
1	U. S. P.[b]	0.750
1	10 ml. NH_4OH 20 ml. ether	0.750
2	10 ml. NH_4OH 20 ml. ether	0.750
3	5 ml. NH_4OH 20 ml. ether	0.697

[a] Eluate evaporated to dryness and heated for fifteen minutes.

[b] U. S. P. Solvent: 8 ml. ammonium hydroxide, 10 ml. alcohol, 20 ml. ether.

Tincture of Belladonna.—To a 10- or 20-ml. portion of the tincture is added enough Stronger Ammonia T. S. to make it alkaline, and the tincture is evaporated to dryness. The residue is dissolved in ether, and the ethereal portion is placed on the column. The assay is completed as above.

Fluidextract of Belladonna.—Ten milliliters of the fluidextract is made alkaline with Stronger Ammonia T. S., and the alkaloids are completely removed by extraction with 3 or 4 portions of chloroform. It is also possible to evaporate the alcohol from the fluidextract before extraction with chloroform; identical results are obtained by either procedure. The chloroform extracts are combined and evaporated to dryness. The residue is dissolved in ether, and the ethereal portion is placed on the column. The assay is complete as above.

DISCUSSION

In order to use an adsorption column, it was necessary to find a method of extraction that would produce a solution suitable for the procedure, and also shorten the time and simplify the official assay. The results obtained by using the U. S. P. maceration and extraction procedures were employed as a comparison standard.

TABLE II.—VALUES OBTAINED BY VARYING THE TIME OF EXTRACTION[a]

Time, Hr.	Alkaloid, %
1	0.509
2	0.736
2½	0.741
3	0.741
4	0.741

[a] Eluate evaporated and heated for fifteen minutes.

TABLE III.—RESULTS OBTAINED BY HEATING THE ELUATE FOR VARIOUS PERIODS

Time, Min.	Alkaloid, %
0	0.750
15	0.741
30	0.741

With these determinations as a guide, it was found possible to cut the maceration period down from overnight, as in the official assay, to one hour (Table I). It was also found that for the maceration, the same results were obtained whether 10 ml. of Stronger Ammonia T. S. and 20 ml. of ether were used, or whether ammonia, alcohol, and ether as in the U. S. P. method. Using a Soxhlet extractor and refluxing for various periods of time (Table II), it was found that it is necessary to extract the drug for two and one-half to three hours for complete extraction. Tests on the exhausted drug proved this to be the case and confirmed the extraction time as stated in the official procedure.

It was also possible to save time, with no decrease in accuracy of results, by evaporating the eluate to dryness and continuing the heating for only fifteen minutes (Table III) instead of the triple evaporation

TABLE IV.—RESULTS OBTAINED WITH AND WITHOUT THE ADDITION OF CHOLINE TO CRUDE DRUG (BELLADONNA LEAVES)

Choline Added, Mg./Ml.	Alkaloid, %
0	0.741
0	0.740
0	0.741
0	0.739
1	0.741
1	0.740
1	0.740
2.2	0.741
2.2	0.741
2.2	0.739

TABLE V

Alkaloid	Added, Mg.	Total Alkaloid Found, Mg.	Alkaloid Recovered, Mg.	Recovery, %
Atropine	0	74.0	...	...
	10	83.9	9.9	99
	10	83.95	9.95	99
	10	84	10.00	100
	33	107	33	100
	33	106.9	32.9	99.7
	33	106.9	32.9	99.7
Hyoscine	10	84	10	100
	10	83.9	9.9	99
	10	84	10	100

has been found in hyoscyamus and belladonna. Choline was therefore added to the powdered leaves before extraction, and the assay was completed as usual. The results were identical with those obtained on the sample to which no choline was added (Table IV). There was not any trace of choline or its degradation products, dimethylamine or trimethylamine, in the residue before titration. When the alkaloidal residue was heated for longer than fifteen minutes, a loss of alkaloid was not observed. Higher results were no doubt due to the fact that there was much less chance of loss of alkaloids since there were fewer manipulations in this procedure. Neither was there a loss due to hydrolysis of the alkaloids.

To insure that the results were reproducible, weighed quantities of the pure alkaloids, atropine and hyoscine, were added to the powdered leaves before extraction. In every case 100% recoveries (Table V) were obtained. Assays were also run on standardized drugs, and the results obtained were 0.03% to 0.1% higher.

The quantity of alkaloids determined by the adsorption column method represents only 1 or 2 Gm. of the drug. By extracting a 10- or 20-Gm. sample of the crude drug, depending on which plant is used, errors due to sampling and weighing of the analytical sample are reduced, although only an aliquot of the extract is used for the determination of the alkaloids.

It was also possible to apply these techniques in the assays of the preparations of the Solanaceae. Duplication of results was excellent using ordinary laboratory techniques. Table VI summarizes these results.

TABLE VI.—SUMMARY OF ASSAYS RUN ON THE SOLANACEOUS DRUGS AND PREPARATIONS

Drug	No. of Samples	U. S. P. Value, Alkaloid, %	Value Alkaloid, Av., %
Belladonna	10	0.59–0.65	0.740
Hyoscyamus	9	0.15–0.17	0.208
Belladonna Root	6	0.69–0.71	0.756
Belladonna Root	8	.....	0.708
Hyoscamus Muticus	7	1.19 (av.)	1.230
Stramonium	6	.....	0.759
Preparations (U. S. P.)			
Powdered Extract of Belladonna	4	.....	1.43
Pilular Extract of Stramonium	6	.....	0.978
Tincture of Belladonna	10	.....	0.0347 Gm./100 ml.
Tincture of Belladonna	6	.....	0.0406 Gm./100 ml.
Fluidextract of Belladonna	8	.....	0.403 Gm./100 ml.

with chloroform as in the official procedure. Final results were the same in both cases. With these modifications and by using an adsorption column to complete the assay, it was possible to complete the assay in less than five hours.

The chlorophyll present in the leaf drugs served as an excellent indicator. The appearance of alkaloid in the eluate coincides with the elution of chlorophyll. The work of Fricke and Kaufman (10) indicated that atropine is not adsorbed by chlorophyll so that it was unnecessary to remove the chlorophyll in the extract.

Because the results from this assay were about 0.03% to 0.1% higher than those obtained by using the U. S. P. method, control tests were run. According to Kunz (11), Tschirch (12), Pictet and Biddle (13), and DeKay and Jordan (14), choline

SUMMARY

By means of selective adsorption it is possible to modify the U. S. P. official assay for solanaceous drugs and their preparations. Time studies on periods of maceration and extraction indicate that maceration for one hour is sufficient, but extraction for three hours is necessary. The assay with these

modifications can be completed in less than five hours, while the official procedure cannot be completed in less than twenty hours.

Precision and accuracy of results are excellent. The techniques required are simple and no complicated equipment is needed.

Results are higher than those obtained by the official assay but recovery experiments indicate that this is not due to the presence of choline or other nitrogenous bases, but rather to the ease of operation and minimal loss of alkaloids.

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Chemical Investigation of *Convolvulus pluricaulis* Chois.*

By N. K. BASU and P. C. DANDIYA

An alkaloid, Sankhpuspine, has been isolated from *Convolvulus pluricaulis*. Also a volatile oil has been obtained by steam distillation of the fresh plant. The isolation of these two principles has been described and their physical and chemical properties have been studied.

Convolvulus pluricaulis Chois. is a hairy perennial herb belonging to the natural order Convolvulaceae. It is distributed throughout the plains of India in the Punjab and eastward to Behar and Chhotanagpur. The herb flowers during the months of March, April, and May. Its common Indian names are Sankhpuspi (Sanskrit), Poprang, Gorakhpinaw, Bephuli, and Dodak. In the Ayurvedic system of medicine, the drug is very extensively used as a brain tonic and in the treatment of some forms of insanity. It is also used in the treatment of sexual neurasthenia.

The plant *Evolvulus alsinoides* belonging to the same natural order is often confused with *C. pluricaulis*. Both the plants grow together in the waste lands and from the outward appearance they look alike although the flowers of *E. alsinoides* are usually blue, while those of *C. pluricaulis* are white to light pink in color.

The first author, with the help of P. P. Lamsal of this laboratory, definitely demonstrated the presence of an alkaloid in the plant. Now the authors have been able to isolate an alkaloid (m. p. 162–164° decomposing at 200°). The plant on steam distillation yielded an essential oil whose physical and chemical constants have been determined.

EXPERIMENTAL

Preliminary extraction with different solvents, soon indicated that the alkaloid is best extracted with hot, rectified spirit. It was also noted that the alkaloidal content of the plant considerably decreases on storage and that prolonged contact with alkalies decomposes the base.

Five kilograms of the freshly collected and dried drug were extracted by soxhlation with hot rectified spirit. After settling for a few days, the major por-

* Received May 22, 1947, from the Department of Pharmaceutics, Benares Hindu University, Benares, India.

tion of the solvent was distilled off and the hot syrupy extract, so obtained, was slowly poured into 2 L. of warm 1% HCl with constant shaking. The mixture was shaken mechanically for two hours, filtered and transferred to a separator. It was then repeatedly shaken with chloroform to remove the coloring matter, each chloroform extract being washed with 1% HCl kept in another separator and the acid washings added to the main bulk. The acid liquid was once again filtered and the filtrate was rendered alkaline with dilute ammonia and shaken repeatedly with chloroform till complete extraction of the alkaloid was effected. The solvent after dehydration on anhydrous sodium sulfate was distilled off from the mixed chloroform extract (500 cc.). The crude alkaloidal residue obtained, weighed 2.8 Gm. It was taken up again in 1% HCl and the above process of purification by shaking out was repeated. The finally obtained reddish brown, alkaloidal residue was further purified as mentioned below.

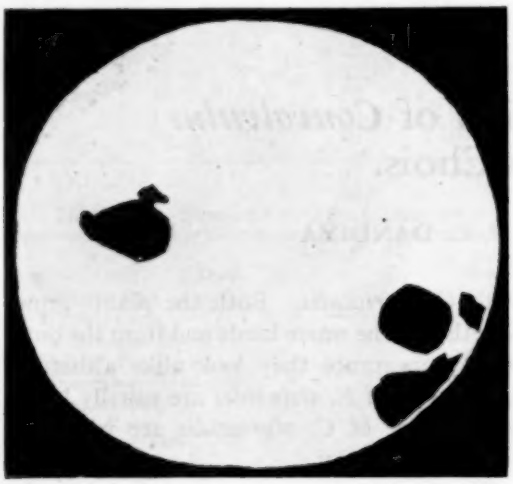

Figure 1

Purification of the Crude Alkaloid.—The crude alkaloid was dissolved in a few cubic centimeters of chloroform and gasoline was added to it drop by drop, whereby a lot of resinous matter precipitated down. The supernatant liquid was decanted off and evaporated on a water bath. The residue so obtained was once again dissolved in a little chloroform and benzene was added dropwise when some more resins separated. The process was repeated successively with ether and petroleum ether. The yellowish alkaloidal residue, as finally obtained was dissolved in a little absolute alcohol, filtered, and crystallized. The crystals were washed with a little ether chloroform mixture to remove the associated resins and recrystallized from absolute alcohol. The total weight of the pure alkaloid so obtained was 60 mg. The base melts at 162–164° (decomposing at 200°). A micro-photograph of the base is shown in Fig. 1.

The name Sankhpuspine is suggested by the authors for the alkaloid. The following derivatives could be prepared:

Picrate	decomposing at 76°
Aurichloride	decomposing at 208°
Platinum chloride	decomposing at 180°

On submitting the base to semimicro combustion the following results were obtained: Carbon, 70.33; Hydrogen, 7.93; Nitrogen, 4.78; Oxygen (by difference), 16.96. Its empirical formula, therefore is $C_{17}H_{23}NO_3$. The empirical weight calculated from the above formula comes to 289.

A few milligrams of the platinum chloride derivative were ignited in a micro-crucible and the residue weighed. The molecular weight, calculated from the data obtained on the assumption that the base is mono-acidic, is 286.

Essential Oil.—By steam distilling 2 Kg. of the fresh flowering plant about 0.2 to 0.3 cc. of oil was obtained floating on the surface of water in the Florentine receiver. By repeating the process, 10 cc. of the oil was collected and the physical and chemical constants were determined. They are given below:

Sp. gr. at 28°	0.8610
Refractive index at 28°	1.4676
Acid value	0.79
Ester value	11.5
Acetyl value	25.6

The oil is pale yellowish having a green tinge. It has a characteristic smell.

CONCLUSION

The alkaloid sankhpuspine (m. p. 162–164°) has been isolated in pure crystalline state from *C. pluricaulis*. Its empirical formula $C_{17}H_{23}NO_3$ has been found out. The fresh plant also contains an essential oil whose physical and chemical constants have been determined.

Chronic Oral Toxicities of Surface-Active Agents*

By O. GARTH FITZHUGH and ARTHUR A. NELSON

Data are given on the effects of the oral administration of alkyl dimethyl benzyl ammonium chloride, sodium dioctyl sulfosuccinate, and sodium lauryl sulfate for two years and the oral administration of sodium lauryl sulfate, sodium dioctyl sulfosuccinate, sodium alkyl aryl sulfonate, polyethylene glycol monoisooctyl phenol ether, and sulfoethyl methyl olylamide for four months. The results of the chronic study indicate a high degree of toxicity for alkyl dimethyl benzyl ammonium chloride. It was toxic to rats at a concentration of 0.063% or more in the diet. Sodium dioctyl sulfosuccinate was slightly toxic at a concentration of 1%. Sodium lauryl sulfate showed no toxic effect at a dosage level of 1%. In the subacute experiment sodium dioctyl sulfosuccinate was toxic at all dosages, sodium lauryl sulfate decreased the growth rate at the 4% concentration, and the other three surface-active agents produced an effect on growth at the 2% concentration.

WITHIN the few years since their introduction the surface-active agents have become very numerous and varied and have found uses in almost all industries (1). Of uses involving possible ingestion by human beings some of these compounds are employed for cleaning and disinfecting bottling plants, restaurants, dairies, and canneries, in the cleaning and peeling of vegetables and fruits for packing, as an ingredient of dentifrices, and in the preparation of pharmaceuticals. Although the surface-active agents can be absorbed through the skin (2) and probably through mucous membranes, the toxicity by oral administration has been most thoroughly studied. Many of these compounds have been studied for toxicity by acute, subacute, or short-term chronic administration in the drinking water or diet of animals. The recent review of Woodard and Calvery (3) gives the available toxicity data from the literature and the acute toxicity data from this laboratory. The present report extends these data to include the chronic oral toxicity of three extensively used surface-active agents and the subacute oral toxicity of three other surface-active agents.

EXPERIMENTAL

Groups of 12 male weanling rats (21 days) from our colony of Osborne–Mendel strain were started on each of 11 diets containing, respectively, 0.25, 0.5, and 1% sodium lauryl sulfate, 0.25, 0.5, and 1% sodium dioctyl sulfosuccinate,[1] 0.063, 0.125, 0.25, and 0.5% alkyl dimethyl benzyl ammonium chloride,[1] and a control with no added ingredient. Ground commercial rat biscuits with 1% added cod-liver oil served as the basic diet. The surface-active agents were mixed with the basic diet by means of a rotary batch mixer. All animals were kept in individual cages in a room with controlled temperature and humidity and were given free access to their respective diets and water. Body weights and food consumption were determined at weekly intervals. Surviving animals were sacrificed at the end of the two-year period.

After it appeared that sodium lauryl sulfate and sodium dioctyl sulfosuccinate were not toxic, or at the most were only slightly toxic, at the 1% level in the diet, a second experiment was begun with higher concentrations of these substances and with three other surface-active agents, *viz.*, polyethylene glycol monoisooctyl phenol ether,[1] sulfoethyl methyl olylamide,[1] and sodium alkyl aryl sulfonate.[1] The purpose of the second experiment was to determine the effect of high dosage levels for a relatively short time. Groups of 5 male weanling rats were started on each of 22 diets containing, respectively, 2, 4, and 8% sodium lauryl sulfate, 2, 4, and 8% sodium dioctyl sulfosuccinate, 0.25, 0.5, 1, 2, 4, and 8% polyethylene glycol monoisooctyl phenol ether, 0.5, 1, 2, 4, and 8% sodium sulfoethyl methyl olylamide, 0.5, 1, 2, and 4% sodium alkyl aryl sulfonate and a control. This experiment was conducted in a manner similar to the first experiment except that all surviving animals were sacrificed at the end of sixteen weeks on the experimental diets.

RESULTS

Two-Year Experiment: Effects on Growth and Mortality.—Growth curves of the experimental groups, with the exception of that for the 0.5% alkyl dimethyl benzyl ammonium chloride, followed

* Received Aug. 27, 1947, from the Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.

[1] These compounds are used under a variety of trade names.

closely that of the controls. The few animals which survived for more than two weeks on the 0.5% dosage of alkyl dimethyl benzyl ammonium chloride grew very slowly. During the fast growing period of the first twelve weeks of the experiment the growth rates of the groups of rats on diets containing 0.25% and 0.125% alkyl dimethyl benzyl ammonium chloride and 1% sodium dioctyl sulfosuccinate were slightly less than that of the control group. These differences in growth rate became more pronounced during the remainder of the first year of the experiment. When the gains in weight of the experimental and control animals during the first year were analyzed (Table I), the differences between controls and the animals on 0.25% alkyl dimethyl benzyl ammonium chloride and 1% sodium dioctyl sulfosuccinate were highly significant ($p. < 0.001$) and the difference between the controls and those on 0.125% alkyl dimethyl benzyl ammonium chloride was significant ($p < 0.05$). Further analyses of the growth data for intervals including the first twelve weeks of the experiment and for the last twenty-six weeks of the first year showed that no other group grew significantly less than the controls either during the fast growing period or during the plateau period. No dislike for the food containing the surface-active agents was evidenced by a decreased food intake in any group of animals with the exception of the group on 0.5% alkyl dimethyl benzyl ammonium chloride. Since this dosage of alkyl dimethyl benzyl ammonium chloride produced extreme diarrhea and bloating of the abdomen, the animals which lived several weeks had a reduced food consumption.

had a gross examination of their tissues, and 71 were examined microscopically. Lung, heart, liver, spleen, pancreas, stomach, small intestine, kidney, adrenal, and testis were sectioned in all instances; colon, thyroid, parathyroid, lymph nodes, leg bones, leg muscles, and bone marrow were sectioned in some. Tissues were fixed in formalin, embedded in paraffin, and stained with hematoxylin-eosin.

Gross and microscopic pathological changes attributable to treatment were seen only in those animals fed alkyl dimethyl benzyl ammonium chloride, and in those chiefly at the highest dosage level of 0.5% of the diet. Nine of that group of 12 rats died or were sacrificed in extremis about one week after beginning feeding. Diarrhea had been noted during life. At autopsy many of these rats showed a moderate to large amount of thin brown, syrupy material in the intestines. In the rat surviving the longest, nine weeks, the cecum was distended with this fluid to about 10 times its normal volume. Microscopically, the stomach showed small foci of mucosal hemorrhagic necrosis, and the lumens of the stomach and intestines contained hemolytic pigment. These microscopic changes were nonspecific but were consistently found, being present in 6 of the 7 rats sectioned from this group. No morphological change in the intestinal mucosa could be made out.

At dosage levels of less than 0.5% of alkyl dimethyl benzyl ammonium chloride, lesions attributable to treatment were seen in only a few animals. One dying after seven weeks on 0.063% had typical lesions as seen at 0.5%. One each at 0.25% and 0.125% died after two weeks with massive pneumonia. One each at 0.25% and 0.063% were sacri-

TABLE I.—MEAN GAIN IN WEIGHT OF MALE RATS GIVEN SURFACE-ACTIVE AGENTS IN THEIR DIET FOR A YEAR

Substance	Dosage, %	No. of Animals	Mean Gain in Weight, Gm.	Standard Error of Mean, Gm.
Sodium lauryl sulfate	0.25	12	454.7	±14.5
	0.5	10	466.0	±15.4
	1	11	467.7	±12.4
Sodium dioctyl sulfosuccinate	0.25	10	436.0	±15.5
	0.5	10	441.3	±14.5
	1	10	395.8	±11.6[a]
Alkyl dimethyl benzyl ammonium chloride	0.063	10	455.5	±21.6
	0.125	10	417.4	±16.4[b]
	0.25	7	297.8	±31.2[a]
Control	...	11	471.9	±13.2

[a] $p < 0.001$. [b] $p < 0.05$.

Only in the case of the group on the 0.5% alkyl dimethyl benzyl ammonium chloride was there a significant difference in mortality from the control group. All of the 12 animals on the diet containing 0.5% alkyl dimethyl benzyl ammonium chloride died within the first ten weeks. At the termination of the experiment the number of surviving animals in each experimental group was not significantly different from that in the control group.

Pathology.—Of the 132 treated and control rats started on the two-year experiments, all but a few ficed because of poor condition after eighty-eight weeks and showed distinct enlargement of the cecum, with watery fluid contents; ascites was present in one animal.

Except as described in the preceding two paragraphs, no gross or microscopic pathological changes resulted from the feeding of alkyl dimethyl benzyl ammonium chloride.

In the animals fed sodium dioctyl sulfosuccinate and sodium lauryl sulfate, there were no changes whatever attributable to treatment.

Four-Month Experiment.—Dosages of polyethylene glycol monoisooctyl phenol ether at 1% or more in the diet decreased the growth rate of rats (Table II). Because of the small number of animals and the individual variation within the group, the difference between the experimental animals at the 1% concentration and the control group was not statistically significant; however, the difference was significant at 2% and highly significant at 4% polyethylene glycol monoisooctyl phenol ether in the diet. Two of the 5 rats on the 4% level and all on the 8% level died within the first week of the experiment. These early deaths appeared to result from a severe irritation of the gastrointestinal tract. Bloating of the intestines and severe diarrhea were observed.

Sulfoethyl methyl olylamide at dosage levels of 4% and 8% significantly retarded the growth rate of rats (Table II). Lower dosages had no effect. Three of the 5 animals on the 8% concentration lived for the duration of the experiment. Gastrointestinal irritation apparently was the cause of death of the 2 rats on this concentration which died during the experimental period.

The tissues of no animal in the four-month experiment were examined microscopically. Gross examinations of all animals showed retardation of

TABLE II.—MEAN GIVEN IN WEIGHT OF MALE RATS GIVEN SURFACE-ACTIVE AGENTS IN THEIR DIET FOR 16 WEEKS

Substance	Dosage, %	No. of Animals	Mean Gain in Weight, Gm.	Standard Error of Mean, Gm.
Sodium lauryl sulfate	2	5	337	±24.8
	4	5	240	±29.3[a]
Sodium dioctyl sulfosuccinate	2	5	220.4	±24.9[b]
Polyethylene glycol monoisooctyl phenol ether	0.25	5	389.6	±22.2
	0.5	5	387.0	±10.5
	1	5	357.6	±17.9
	2	5	319.4	± 9.7[a]
	4	3	200.3	±18.0[b]
Sulfoethyl methyl olylamide	0.5	5	364.6	±15.6
	1	5	361.2	± 7.5
	2	5	333.3	±10.3
	4	5	332.0	± 8.1[a]
	8	3	272.3	±10.9[a]
Sodium alkyl aryl sulfonate	0.5	5	379.0	±11.9
	1	5	351.0	±21.9
	2	5	205.4	±23.9[b]
Control	..	5	393.0	±22.6

[a] $p < 0.05$. [b] $p < 0.001$.

All dosages of sodium dioctyl sulfosuccinate used in the four-month experiment were very toxic. Although all animals in the group on the 2% concentration of sodium dioctyl sulfosuccinate lived for the duration of the experiment, there was a marked retardation of growth (Table II). Only one animal in the group on 4% lived for the duration of the experiment and it grew very slowly. All animals on the 8% dosage level died within the first week with severe gastrointestinal symptoms.

The 4% concentration of sodium lauryl sulfate significantly retarded the growth of rats (Table II). The rats on the 2% sodium lauryl sulfate were slightly smaller than the controls; however, the difference in the mean gain in weight of the experimental animals and that of the controls was not significant. The rats on the 8% level died within the first two weeks with severe diarrhea and bloating of the intestines.

The analysis of the mean gain in weight (Table II) showed a slight retardation of growth for the rats on 1% sodium alkyl aryl sulfonate and a highly significant effect on the 2% dosage level. The animals on the 4% concentration in the diet grew very little and died within the first week of the experiment. Severe bloating and diarrhea occurred before death in all animals on 4% sodium alkyl aryl sulfonate.

growth and gastrointestinal irritation by the higher dosages of each surface-active agent. No other gross lesion attributable to treatment was seen in any animal.

SUMMARY

In a two-year chronic toxicity experiment alkyl dimethyl benzyl ammonium chloride was the most toxic of three surface-active agents. It was toxic to rats at a concentration of 0.063 per cent or more in the diet. At a concentration of 0.5 per cent all animals died within ten weeks; at a concentration of 0.063 per cent two animals showed histopathological lesions and the growth rate of the group was slightly less than that of the controls. The degree of toxicity of other dosages was related to the concentration of the alkyl dimethyl benzyl ammonium chlo-

ride in the diet. The 1 per cent concentration of sodium dioctyl sulfosuccinate produced a significant retardation of growth rate but no gross or histopathological lesion. Sodium lauryl sulfate was not toxic at the dosage levels of 1 per cent or less in the diet.

In a four-month experiment 4 per cent sodium lauryl sulfate in the diet produced a significant decrease in growth of rats. Sodium alkyl aryl sulfonate, polyethylene glycol monoisooctyl phenol ether, and sulfoethyl methyl olylamide produced similar growth effects at the 2 per cent concentration. Death occurred in all animals on 4 per cent sodium alkyl aryl sulfonate, on 8 per cent sodium dioctyl sulfosuccinate, and on 8 per cent sodium lauryl sulfate. Three of the 5 animals on 8 per cent sulfoethyl methyl olylamide lived for the duration of the experiment.

The toxic effects of the surface-active agents studied in the experiments were produced by irritation of the gastrointestinal tract. To an extent which depended on the concentration of the surface-active agents in the diet, this irritation prevented proper nutrition. In severe cases of irritation death resulted.

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The Effect of Folic Acid Analogues on the Action of Dopa Decarboxylase*

By GUSTAV J. MARTIN and J. M. BEILER

The inhibitory effect of a series of folic acid displacing agents on dopa decarboxylase is reported. An attempt is made to correlate structure and function in these compounds.

EXPERIMENTAL results obtained by various workers in the past few years have shown that relatively slight structural changes in many compounds which have metabolic functions may result in the formation of new compounds which are metabolically antagonistic to those from which they are derived. Generalizations have been made (1, 2) about the kinds of structural changes which will produce corresponding functional changes. It has been pointed out, however (1), that these generalizations are nothing more than that, and that often structural changes which are expected to produce metabolic antagonists fail to do so.

With this in mind, an analysis of the structural changes in any given compound, which will result in the formation of effective antagonists to this compound, becomes of more than theoretical interest; it must at least provide additional data for consideration and may at best indicate a point of departure for the synthesis of displacing agents of greater and greater effectiveness.

It has been reported (3) that two folic acid displacing agents—7-methyl folic acid (4) and N-[4-{[(2-amino-4-hydroxy-6-pteridyl)methyl]amino}benzoyl] aspartic acid (pteroyl aspartic acid) (5)—were effective in inhibiting the decarboxylation of dihydroxy *L*-phenylalanine by dopa decarboxylase. The action of several other folic acid analogues on this enzyme was assayed, in the hope of obtaining some correlation between structure and function.

The compounds tested included 7-methyl pteroyl aspartic acid (5); 7-methyl pteroic acid (5); 2-oxyfolic acid (5); 2-oxypteroic acid (5); and N-{4-[(4-quinazoline)amino] benzoyl}glutamic acid (6) (Formula I).

* Received Aug. 15, 1947, from the Research Laboratories of the National Drug Company, Philadelphia, Pa.

EXPERIMENTAL

The method used for assay was the same as that previously reported (3). The enzyme was prepared from rat kidney by the method of Holtz and Credner (7), and its activity measured by the determination in the Warburg apparatus of the amount of CO_2 evolved by the decarboxylation of the substrate. The difference in the volumes of CO_2 given off in the presence and absence of the various compounds tested was taken as a measure of their inhibitory action.

The results obtained, including those previously reported, are presented below in tabular form.

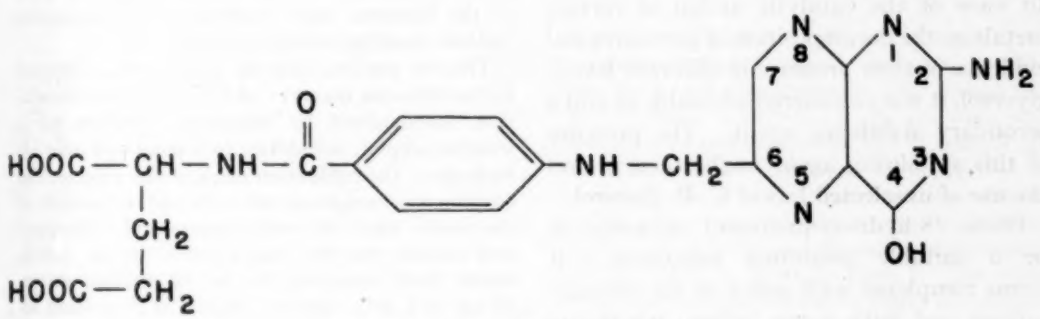
second type the glutamic acid moiety has been either substituted for or omitted. The third type represents a combination of the first two. Finally, in the fourth type the pteridyl grouping itself has been substituted for.

The results show that the most effective displacers of those tested have a substituent on the pteridyl part of the molecule, in combination with an amino acid. Substitution of aspartic acid for glutamic acid does not change significantly the inhibitory effect.

Substitution of aspartic acid for glutamic without the presence of the substituent on the pteridyl ring,

TABLE I.—EFFECT OF FOLIC ACID ANALOGUES ON ACTION OF DOPA DECARBOXYLASE

Substance	Concentration, γ/Cc.	Difference in Structure from Folic Acid	Inhibition, %
7-Methyl folic acid	30	Methyl group at 7	25
Pteroyl aspartic acid	30	Glutamic acid replaced by aspartic acid	15
7-Methyl pteroyl aspartic acid	30	Glutamic acid replaced by aspartic, plus methyl group at 7	30
7-Methyl pteroic acid	30	Methyl group at 7. No glutamic acid	15
2-Oxyfolic acid	30	Amino group at 2 replaced by OH	..
2-Oxypteroic acid	30	Amino group at 2 replaced by OH. No glutamic acid	..
Quinazoline compound	3000	Replacement of ring system with quinazoline ring	0



Formula I—Pteroyl Glutamic Acid

N[4{[(2-Amino-4-hydroxy-6-pteridyl)methyl]amino} benzoyl]glutamic acid

No figures are given for the two oxy-compounds tested. Although at the concentrations noted there was an inhibitory effect, it is felt to be uncertain whether this is specific. At higher concentrations both these compounds had a toxic effect, probably due to a denaturation of the protein of the enzyme, and caused an actual uptake of gas in the manometers. At lower concentrations, therefore, it is not known whether the figures obtained represented the actual amount of gas given off, or were a resultant of the gas given off and that taken up. This effect was not obtained with any of the other compounds tested.

The substances listed above fall into four types as regards their chemical differences from folic acid. The first type includes those compounds which differ by the presence of an additional or substituted group on the pteridyl part of the molecule. In the

or complete removal of the amino acid, results in the formation of a less effective displacing agent.

Replacement of the pteridyl ring with a quinazoline ring does not produce an effective displacing agent. In fact, this compound has been reported actually to have folic acid activity (6).

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The Effect of Oxine (8-Hydroxyquinoline) on the Stability of Glycerite of Hydrogen Peroxide*

By ETHAN ALLAN BROWN, WILFRED B. KRABEK, RITA E. SKIFFINGTON, and GEORGE A. CRUICKSHANK

Oxine (8-hydroxyquinoline) has been found to improve the stability of urea peroxide and of hydrogen peroxide in solutions of these drugs in glycerol.

THE USE OF glycerol in varying percentages for the stabilization of aqueous peroxide solutions has been reported in monographs by Kausch (6) and by Machu (7). The stability of urea peroxide in substantially anhydrous glycerol has been reported by Brown and his collaborators (1).

While testing the bacteriotoxic action of glycerite of hydrogen peroxide as derived from urea peroxide (2, 3) it was found that, although some lots of the solution were relatively stable, other lots decomposed rapidly. In view of the catalytic action of certain metals on the decomposition of peroxides and evidence of their presence in different lots of glycerol, it was considered advisable to add a secondary stabilizing agent. The presence of this stabilizing agent might then permit the use of unselected lots of C. P. glycerol.

Oxine (8-hydroxyquinoline) appeared to be a suitable inhibiting substance. It forms complexes with many of the metallic cations and with some anions, which are practically un-ionized and quite insoluble. In small amounts, the compounds formed appear to remain suspended in the glycerol solution. In addition, it has a considerable bacteriotoxic activity of its own, although the tendency is to be bacteriostatic rather than bactericidal (2, 3). In glycerol which is relatively free of heavy metals, oxine produces a yellowish green to deep amber solution. This color tends to absorb actinic light of the blue end of the spectrum, thus decreasing the decomposition of the peroxide solution caused by ultraviolet light.

Examination of the literature shows that oxine has been suggested as a stabilizer for aqueous solutions of peroxide (4–8). In several cases it was used in conjunction with formalin. In Germany it (5, 8) was used as a stabilizer for concentrated hydrogen peroxide intended for rocket fuel purposes. Its use as a stabilizer for glycerol solutions of hydrogen peroxide does not appear to have been previously suggested.

* Received July 9, 1947.

EXPERIMENTAL

For the experiments at 50°, urea peroxide was dissolved in C. P. glycerol to give a concentration of approximately 4%. Concentrated hydrogen peroxide (50% by weight) was diluted with C. P. glycerol to approximately 1.46% (corresponding to 4% urea peroxide). For room temperature experiments, corresponding solutions were used. Two other solutions were made up from the same lots of urea peroxide and glycerol, one containing 0.1% and the other 0.05% oxine. Aliquot parts of each of the solutions were titrated by an iodometric method described briefly as follows.

Transfer pipettes (Kimble 2-ml.) were calibrated in the following manner: A peroxide-glycerol solution, standardized by iodometric titration of a weighed aliquot, was drawn to a point not over 1/8 inch above the calibration mark of the pipette, the solution was brought to the mark and the outside of the pipette wiped dry with a paper towel. The glycerol solution was then blown into a 500-ml. Erlenmeyer flask containing 100 ml. of distilled water, 10 ml. of a 20% aqueous solution of potassium iodide, and 6 ml. of diluted hydrochloric acid (1:1) containing 26 ml. of a 2% ammonium molybdate per liter. The flask was swirled during delivery of the aliquot solution. The pipette was then rinsed five times with distilled water by transferring approximately 2 ml. each time from a 25 × 150-ml. test tube containing about 50 ml. of water. It was then rinsed thrice more by drawing and expelling water from the test tube into the pipette and into the test tube. The contents of the tube were then quantitatively transferred to the flask containing the aliquot peroxide-glycerol solution. After standing five minutes in the dark, the liberated iodine was titrated with 0.1 *N* thiosulfate solution.

This technique has, in the hands of reasonably careful operators, shown a reproducibility of 0.05 ml. or less of 0.1 *N* thiosulfate solution. This corresponds to 0.000235 Gm. of urea peroxide or approximately 0.26% of the absolute amount of urea peroxide present in the aliquot solutions. A more complete description of the method is being submitted elsewhere for publication.

Kimble pipettes were found more satisfactory than some other brands because of the slightly larger orifice. Squibb U. S. P. potassium iodide has been found as satisfactory as C. P. grades of some other brands, since 20% solutions of this grade, when stored in the dark for periods up to one week, gave no blank in twenty minutes. Each lot should, however, be controlled in this respect. Small double action rubber bulbs or a water aspirator, followed by a small rotary air compressor to expel the fluid, has been found suitable for the rapid transfer of the glycerol solutions.

After the initial titration of aliquot portions of the solution, the latter were placed in an Elconap incubator set at 50° for the high-temperature test. Aliquot parts were removed periodically for titration in duplicate. The values obtained for urea peroxide are shown in Fig. 1; for hydrogen peroxide in Fig. 2. In the discussion of the figures and tables, it is understood that unselected lots of C. P. solution constituents were used.

With 0.1% Oxine at Room Temperature
Without Oxine at Room Temperature
Without Oxine at 50°
With 0.1% Oxine at 50°
With 0.05% Oxine at 50°
Residual Peroxide in % of Original Amount
DAYS

Fig. 1.—Stability of urea peroxide-glycerol solutions with and without oxine.

A number of factors appear to affect the stability of the solutions, particularly those without oxine. Among these factors are light, temperature of storage, lots of constituents, volume of the solution, the peroxide content, and the method of preparation of the solution.

At room temperature, the effect of the color of the container has a marked effect on the peroxide-glycerol solutions in the absence of oxine. When exposed to diffuse daylight, these solutions tend to break down more rapidly in a clear glass bottle than

With Oxine at Room Temp.
With Oxine at 50°
Without Oxine at Room Temp.
Without Oxine at 50°
Residual Peroxide in % of Original Amount
DAYS

Fig. 2.—Stability of hydrogen peroxide-glycerol solutions with and without oxine.

in a container of amber glass. Some lots, however, show much less decomposition than others (1). This variability is particularly marked when solutions prepared from unselected lots of glycerol and of urea peroxide are compared. In the presence of oxine, the color of the container has much less effect, the yellow color of the oxine apparently acting, in part at least, to filter out the actinic wave lengths. This light effect is shown in Table I. The values shown were unselected except in regard to completeness of record and similarity of conditions.

TABLE I.—THE EFFECT OF OXINE AND OF TYPE OF CONTAINER ON STABILITY OF GLYCERITE OF HYDROGEN PEROXIDE SOLUTIONS

Lot No.	Age of Solution in Days	Ml. 0.1 *N* Thiosulfate[a] Amber Glass	Clear Glass
	Without Oxine		
40	629	15.52	1.40
39	640	15.50	1.09[b]
43	576	16.50	3.79[c]
44	576	28.09	26.40[d]
60	293	17.32	0.00
	With 0.1% Oxine		
4	528	16.02	16.23
5	516	16.10	16.09
7	496	16.70	16.60
1[e]	371	18.05	16.09

[a] A 4% solution of urea peroxide required 21.60 cc. 0.1 *N* thiosulfate under the experimental conditions.
[b] Aliquot from clear glass jug required 17.25 ml.
[c] Aliquot from clear glass jug required 19.20 ml.
[d] Aliquot from amber glass jug required 35.53 ml. (This was originally an 8% solution.)
[e] Original titer for this solution was not available.

Of 37 different solutions of urea peroxide in glycerol containing 0.1% oxine, not one has broken down to the extent of 30% over periods ranging up to 630 days. Of 28 solutions without oxine, but also stored in amber glass at room temperature, 9 have decomposed 50% or more. If solutions stored in clear glass containers are included, the statement regarding solutions containing oxine remains true, but of 44 different solutions containing no oxine, 25 have decomposed at least 50% in 630 days or less, generally in less than one year.

The effect of the volume of the solution is variable, but in general a small volume of the solution tends to break down more rapidly than a larger volume, under random methods of preparation. This also is indicated in Table I, from which it will be noted that the solution in jugs appeared to be more stable than in smaller clear glass bottles. This appears also to be true when the containers are amber in color.

At 50°, the effect of the addition of oxine is quickly evident, as Fig. 1 indicates. The constituents of these three urea peroxide solutions studied were the same lots and were treated in the same manner with the exception of the oxine content. In this experiment, the urea peroxide solution containing oxine (0.1%) still showed a trace (0.5%) of the original amount present after 247 days at 50°. Other tests have shown results of the same order. It is of interest to note the nearly straight line relationship between residual peroxide and time. The curve actually is slightly convex for the first part and slightly concave for the second part. The curve for the urea peroxide-glycerol solutions without oxine tends to be sigmoid in shape. This shape appears to be quite characteristic of solutions containing the two constituents. The addition of anticatalysts tends to straighten out the S-shaped curve to one more closely approximating a straight line, as the efficiency of the anticatalyst increases.

When hydrogen peroxide is used in place of urea peroxide, the results are somewhat different. In the absence of oxine, the decomposition is more rapid than with urea peroxide. In the presence of 0.1% oxine and a temperature of 50°, the hydrogen peroxide-oxine solution is somewhat more stable than the solution containing urea peroxide and oxine, as shown in the second table.

At room temperature, the hydrogen peroxide solution without oxine gave a test for the peroxide up to thirty days, although the bulk of the peroxide had decomposed at the end of twenty days. Addition of oxine resulted in a solution which had lost approximately 5% in 199 days. Under the same conditions, urea peroxide without oxine had lost 20% in sixty-three days and the urea peroxide solution, containing 0.1% oxine, still retained 93% at the end of 203 days.

SUMMARY

Oxine (8-hydroxyquinoline) markedly improves the stability of glycerol solutions of urea peroxide and of hydrogen peroxide, both at room temperature and at 50°. It decreases the decomposition of the solutions when stored in clear glass containers in diffuse daylight, nearly to the rate of decomposition of solutions stored in amber containers.

When residual peroxide content (at 50°) is plotted against time on coordinate paper, the rate of decomposition of solutions containing oxine is nearly constant over the greater part of the resulting curve. Under the same conditions, urea peroxide in glycerol, without oxine, shows a rapid decomposition at 50° resulting in a curve of sigmoid shape.

At room temperature, none of 37 solutions containing oxine, and stored in amber glass, broke down to the extent of 30 per cent over a period of time ranging up to 630 days. Of 28 solutions without oxine, 9 lost 50 per cent or more of their original peroxide content in the same period.

Under the experimental conditions, small volumes of peroxide-glycerol solutions appear to break down more rapidly than large volumes.

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Chlorobutanol Assay*

By HOWARD JENSEN† and PAUL JANNKE‡

Chlorobutanol can be assayed by decomposing it with standard alkali solution and back-titrating the residual alkali. Chlorobutanol in oil solutions is assayed without extraction from the oil by hydrolysis of the compound, distilling with the aid of water vapor, and determining the acetone in the distillate by iodometric method.

IN RECENT years, chlorobutanol has come into prominence as an antiseptic and bacteriostatic in ampul preparations and as a preservative for glandular products. In most of these cases, it is used in concentrations of from 0.1 to 0.5 per cent.

Though chlorobutanol has been known for some time and has been official in the last three revisions of the U. S. P., the monograph has never included an assay for this substance. Since it is widely used in parenteral preparations, it would seem that an assay method for the compound would be highly desirable.

Various methods have been suggested for the assay of chlorobutanol, though some are detailed or cumbersome. All determinations are based upon the hydrolysis of the compound with alcoholic or aqueous alkali into inorganic halides and acetone, followed by the quantitative estimation of either hydrolysate.

In 1937, Rosenthaler (1) reported that chlorobutanol decomposes upon warming with alkali into its components, *viz.*, acetone and chloroform. Prior to this, Sinton (2) had proposed two methods for assaying chlorobutanol, one based on the argentimetric determination of the halide formed by hydrolysis with potassium hydroxide, and the second based on the iodometric determination of the acetone liberated in the hydrolysis reaction with sodium hydroxide. Later, Sinton (3, 4) reported on the adaptation of the argentimetric method to ampul solutions from which the chlorobutanol was steam distilled prior to its determination. In 1941, Cowles (5) reported further on the methods described.

Attempts to determine the quantity of chlorobutanol in ampul solutions lead to many new problems. Ampuls containing chlorides or proteins preclude the use of determinations employing the silver halides. On the other hand, oily solutions or suspensions are capable of absorbing iodine, thus nullifying the acetone-type of reaction.

EXPERIMENTAL

Pure Chemical.—Khait (6) described the quantitative hydrolysis of chloral hydrate with an excess of standard sodium hydroxide solution followed by back-titration of the excess alkali with standard acid, in the presence of phenolphthalein T. S. This method was applied in principle to chlorobutanol. The sample (about 200 mg., accurately weighed) contained in a 250-cc. flask was refluxed for fifteen minutes with 25 cc. of 0.5 *N* alcoholic potassium hydroxide solution. After cooling, distilled water and phenolphthalein T. S. were added, and the excess of alkali was titrated with 0.5 *N* acid solution. In all of the assays performed in this manner, the results corresponded to from 105% to 110% of the amount of chlorobutanol actually present. When methyl orange T. S. was used, the results were favorable. To prove the point in question, samples and a blank were titrated to the phenolphthalein end point, and then methyl orange T. S. was added and titration was continued to the end point for this indicator. The amount of acid which would have been required as of each indicator is shown in Table I.

TABLE I

Indicator	Sample No. 1, Cc.	Sample No. 2, Cc.	Blank, Cc.
Phenolphthalein	27.10	26.05	33.62
Methyl orange	28.15	27.20	34.14
Difference	1.05	1.15	0.52

This discrepancy in titration figures may be explained by the presence of small amounts of carbonate in the alkali used (see blank) and by the partial oxidation of carbon monoxide formed in the hydrolysis of chlorobutanol (see Discussion). The carbon dioxide thus produced combines with the hydroxide forming carbonate which would give a false end point with phenolphthalein at the bicarbonate stage. When methyl orange was used, however,

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both the hydroxide and the carbonate were titrated and the true amount of hydroxide consumed in the reaction could be determined with the aid of a blank.

$$(CH_3)_2C(OH)CCl_3 + \underset{3KOH}{\text{excess}} \rightarrow 3KCl + \ldots \text{ (see Discussion)}$$

$$\underset{KOH}{\text{residual}} + HCl \xrightarrow{M.O.} KCl + H_2O$$

Therefore, each cc. of 0.1 *N* potassium hydroxide consumed is equivalent to 0.0059143 Gm. anhydrous chlorobutanol.

Using methyl orange T. S. as indicator the assay described was conducted on a series of samples with the results shown in Table II.

TABLE II

Sample No.	Chlorobutanol Present, Gm.	Chlorobutanol Found, Gm.	Chlorobutanol Actual Amount, %
1	0.2441	0.23922	98.7
2	0.1877	0.18692	100.4
3	0.2178	0.21656	99.4
4	0.1683	0.1707	98.6
5	0.2013	0.1993	99.0
6	0.2066	0.2012	97.4
7	0.2026	0.1993	98.3
8	0.2285	0.2328	101.8
9	0.2056	0.2047	99.6
10	0.2100	0.2101	100.0

Ampul Solutions.—To avoid the difficulties involved in the isolation and the recovery of chlorobutanol from ampul solutions, the following method was devised, based on observations from several experiments:

A sample containing 50 mg. of chlorobutanol (10 cc. of a 5% solution in peanut oil) was transferred by means of a pipette to a Kjeldahl flask which contained 200 cc. of a solution of 3 Gm. of potassium hydroxide and 3 Gm. of calcium hydroxide. Two cubic centimeters each of Fehling's solutions A and B were added, and with the trap in place the mixture was allowed to stand with occasional agitation for thirty minutes. The distillation was then conducted with an adapter on the condenser dipping beneath the surface of 50 cc. of water contained in a 500-cc. glass-stoppered flask which was immersed in a freezing mixture. Between 100 and 150 cc. of distillate were collected, and then 25 cc. of 1 *N* sodium hydroxide and 25 cc. of 0.1 *N* iodine solutions were added. The flask was then removed from the ice bath and allowed to stand for fifteen minutes at room temperature. After adding another 10-cc. portion of 0.1 *N* iodine solution, the mixture was acidified with 1 *N* hydrochloric acid and the excess of iodine was titrated with 0.1 *N* sodium thiosulfate solution in the usual manner. A blank is necessary to determine the amount of iodine consumed.

TABLE III

Sample No.	Chlorobutanol Present, Gm.	Chlorobutanol Found, Gm.	Chlorobutanol Actual Amount, %
1	0.05133	0.05245	102.1
2	0.05133	0.05129	99.9
3	0.05133	0.05300	103.2
4	0.0430	0.04324	100.5
5	0.0565	0.05726	101.2
6	0.0579	0.05703	98.5
7	0.0554	0.05457	98.5
8	0.0574	0.05703	99.4
9	0.0563	0.05752	102.0
10	0.0598	0.05998	100.2
11	0.0543	0.05328	98.1
12	0.0476	0.04804	100.9

$$(CH_3)_2C(OH)CCl_3 + 3KOH \rightarrow CH_3COCH_3 + \ldots \text{ (see Discussion)}$$

$$CH_3COCH_3 + 3I_2 \rightarrow CHI_3 + CH_3COOK + 3KI + 3H_2O$$

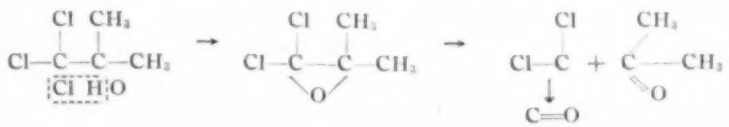
therefore $3I_2 = (CH_3)_2C(OH)CCl_3$

Therefore, each cc. of 0.1 *N* iodine is equivalent to 0.002957 Gm. anhydrous chlorobutanol.

A series of 12 determinations for chlorobutanol in peanut oil, conducted in the manner described, gave the results indicated in Table III.

DISCUSSION

It has been suggested previously that the decomposition of chlorobutanol by alkali does not yield acetone and chloroform only. Mossler (7) reported in 1909 that the action of alkali in alcohol on chloroform produces ethylene, formic acid, and carbon monoxide. The formation of the unstable carbon dichloride (CCl_2) by the abstraction of hydrogen chloride from chloroform offers an explanation for the formation of carbon monoxide and of ethylene. Bressanin and Segre (8) observed that when chlorobutanol is treated with 10% potassium hydroxide solution, 1 mole of carbon monoxide is liberated. The reaction is represented as:



Early in these studies it was observed that some reducing agent was interfering with the efficiency of the reaction, giving results up to 110%. It was assumed to be carbon monoxide, as would be concluded from the literature reports. Several oxidizing agents, such as potassium permanganate and hydrogen peroxide, were added to the digestion mixture with varying degrees of success, but Fehling's solution gave the best results.

The mixture of calcium and potassium hydroxides

provided ideal conditions for conducting the distillation after digestion. The soluble soaps formed by potassium hydroxide alone foamed over and ruined the assay; whereas calcium hydroxide alone gave low results consistently, due either to incomplete hydrolysis of the chlorobutanol or to the occlusion of unreacted chlorobutanol within the insoluble soaps which are formed in large masses.

The iodoform reaction was found to be more complete if the last 10 cc. of iodine solution were added after allowing the distillate to warm to room temperature, rather than adding all of the reagent at once to the cold distillate.

SUMMARY

Methods have been described in detail for the assay of chlorobutanol in the free state and in oil solution.

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Notes

A Note on the Synthesis of Veratric Acid*

By CARL J. LINTNER and LLOYD M. PARKS

THE RECENT need in this laboratory for a quantity of veratric acid (3,4-dimethoxybenzoic acid) prompted a review of the literature for methods of synthesis of this compound and the investigation of a simpler method which suggested itself from the literature survey.

Veratric acid has been prepared by several different methods, among which may be mentioned the oxidation of eugenol methyl ether with potassium dichromate (1), with potassium permanganate (2); oxidation of isoeugenol methyl ether with potassium dichromate (3); methylation of proto-catechuic acid with methyl alcohol (4), with dimethyl sulfate (5), with diazomethane (6); and finally, the oxidation of veratraldehyde with potassium dichromate (7), with potassium hypobromite (8), and with potassium permanganate. Using the last-named method Perkins and Edwards (9) reported a 91% yield of the impure product while Raiford and Perry (10) obtained an 80% yield of recrystallized product.

Pearl (11) has recently reported that vanillin is oxidized in substantially quantitative yields to vanillic acid by means of alkali and one-half mole of silver oxide, a reaction which he later showed (12) to be generally applicable for the preparation of ortho and para hydroxy substituted aromatic acids from the corresponding aldehydes. His method has been applied with good results to the preparation of veratric acid. With vanillin as the starting product, two alternate procedures were used: (*a*) methylation of vanillin to veratraldehyde which was isolated and oxidized to veratric acid, and (*b*) oxidation of vanillin to vanillic acid which was then methylated, without isolation and in the same reaction flask, to veratric acid.

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EXPERIMENTAL[1]

Veratraldehyde—The veratraldehyde used was prepared by methylation of vanillin by the method of Barger and Silberschmidt (13), giving a product (89% yield), m. p. 43.5°, which was used without further purification.

Oxidation of Veratraldehyde with One-Half Mole of Silver Oxide.—A solution of 8 Gm. (0.2 mole) of sodium hydroxide in 75 cc. of water was added to 34 Gm. (0.2 mole) of silver nitrate in 175 cc. of water, the precipitated silver oxide stirred, collected on a filter, and washed free of nitrates. After transferring the moist silver oxide to a 1-L. three-necked reaction flask and covering with 200 cc. of water, 40 Gm. (1.0 mole) of solid sodium hydroxide was added, the temperature adjusted to 50–55° by means of a heating mantle, and with constant stirring 33.23 Gm. (0.2 mole) of veratraldehyde was added in one portion. As the reaction set in the silver oxide was

[1] All melting points are corrected.

reduced to metallic silver and the temperature of the reaction mixture rose to 80–85°, at which point outside heat was discontinued. Filtration of the mixture, washing, and drying of the precipitate gave 21.3 Gm. (99%) of metallic silver. Upon acidification of the combined filtrate and washings with sulfur dioxide a white crystalline precipitate separated. This was collected on a filter, washed with water, and dried to give 27.8 Gm. (76.3%) of veratric acid, m. p. 180.5–181°. Ether extraction of the filtrate yielded an additional 0.91 Gm. (2.5%) of veratric acid, m. p. 178.5–180°. The melting point of a mixture with a sample of veratric acid, prepared by oxidation of veratraldehyde with potassium permanganate, was not depressed.

Oxidation of Veratraldehyde with Alkali and One Mole of Silver Nitrate.—In a 1-L. three-necked flask, equipped with mechanical stirrer and reflux condenser, 33.23 Gm. (0.2 mole) of veratraldehyde was melted by means of a heating mantle. With vigorous stirring 48 Gm. (1.2 mole) of sodium hydroxide in 100 cc. of water was added and the temperature of the reaction mixture adjusted to 50–55°. With constant stirring a solution of 34 Gm. (0.2 mole) of silver nitrate in 160 cc. of water, previously warmed to 55°, was added from a separatory funnel at the rate of approximately 3 drops per second. As reaction set in, the silver oxide, momentarily formed as a black powder, was reduced to metallic silver, the temperature rising to approximately 80°, at which point outside heat was discontinued. After addition of the silver nitrate solution the reaction mixture was filtered while hot and the precipitate washed with water. Drying of the precipitate yielded 21.465 Gm. (99.4%) of metallic silver. The combined filtrate and washings, upon cooling and acidifying with sulfur dioxide, yielded a white crystalline precipitate. This was filtered and dried at 90°, yielding 34.35 Gm. (94.3%) of veratric acid, m. p. 180.5–181°. Ether extraction of the filtrate yielded an additional 1.02 Gm. (2.8%) of product, m. p. 178.5–180°. The melting point of a mixture with authentic veratric acid was not depressed.

Oxidation of Vanillin with Alkali and One Mole of Silver Nitrate and Methylation of the Resulting Product.—To a 1-L. three-necked flask, equipped with mechanical stirrer and reflux condenser, were added 30.43 Gm. (0.2 mole) of vanillin, 100 cc. of boiling water, and 48 Gm. (1.2 mole) of sodium hydroxide in 400 cc. of water, and the temperature adjusted to 50–55° by means of a heating mantle. With constant stirring a solution of 34 Gm. (0.2 mole) of silver nitrate in 160 cc. of water, previously warmed to 55°, was added from a separating funnel at the rate of 2–3 drops per second. The reaction proceeded in the same manner as noted above; after addition of the silver nitrate solution the reaction mixture was filtered while hot and the precipitate washed with water.

The combined filtrate and washings were placed back in the reaction flask, and immersed in an ice bath. When the temperature reached approximately 10°, 25.23 Gm. (0.2 mole) of dimethyl sulfate was added from a separatory funnel, slowly and with constant stirring. After addition of this portion the stirring was continued for twenty minutes and then an additional 12 Gm. (0.1 mole) portion of dimethyl sulfate added in the same manner and the stirring continued for an additional 10 minutes. At the end of this time the contents of the reaction flask were refluxed for two hours, 20 Gm. of sodium hydroxide in 30 cc. of water added, and refluxed for an additional two hours in order to saponify any ester which may have been formed. The reaction mixture was cooled and acidified with sulfur dioxide to give a white crystalline precipitate. This was filtered, washed, and dried to yield 30.27 Gm. (83.1%) of veratric acid, m. p. 179.5–180°, as white crystals with a yellow tinge. Ether extraction of the filtrate yielded an additional 1.86 Gm. (5.1%) of product, m. p. 178.5–179.5°. On decolorizing with Norit and recrystallizing from hot water white crystals, m. p. 180.5–181°, were obtained. The melting point of a mixture with authentic veratric acid was not depressed.

SUMMARY

Veratric acid has been prepared by the alternate procedures of (*a*) oxidizing veratraldehyde with alkali and one-half mole of silver oxide or (*b*) with alkali and one mole of silver nitrate, and (*c*) oxidizing vanillin with alkali and one mole of silver nitrate and methylating the resulting vanillic acid without isolation. Of these, procedure *b* gave the purest product in good yields (86.4% overall from vanillin as the starting product); procedure *c* gave the best yield (88.2% from vanillin as the starting product) and had an added advantage in that the intermediate vanillic acid did not need to be isolated, but it did not give as pure a product as procedure *b*.

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Microscopic Identity Tests for Hydrastis and Its Principal Alkaloids, Hydrastine and Berberine*

By GEORGE L. KEENAN†

ONE of the identity tests for *Hydrastis* relies upon moistening the rhizome or root with water and mounting it in sulfuric acid, resulting in the formation of acicular crystals, presumably the sulfates of the alkaloids. The other test recommends the use of filtered ultraviolet light in which the broken surfaces of the rhizome or root exhibit a brilliant fluorescence (1). The acicular crystals produced with sulfuric acid are more distinctly visible when the preparation is examined with crossed nicols, the extinction on the needles being parallel and the sign of elongation negative. Hydrochloric acid will also produce the needles, apparently as the hydrochlorides of the alkaloids.

The purpose of this paper is to call attention to the effect produced with concentrated nitric acid, a color reaction which is quite as distinctive for identification purposes as the tests already referred to. Also, attention is called to the optical-crystallographic properties and microchemical reactions of the principal alkaloids of *Hydrastis*, hydrastine, and berberine.

When a drop of concentrated nitric acid is applied to the unground rhizomes or roots of *Hydrastis*, or to the powdered material, a distinct reddish brown color is formed. Incidentally, barberry root (*Berberis vulgaris, L.*), gives the same color test, and it would also be expected in the genera of other plant families such as the Ranunculaceae, Rutaceae, and Papaveraceae, in which berberine has been reported as one of the constituent alkaloids. Berberine alkaloid gives the same color test, but hydrastine does not.

The identity tests for the two principal alkaloids of *Hydrastis* rely upon the optical-crystallographic properties and microchemical reactions that are significant for berberine and hydrastine. Since both of the alkaloids are crystalline, they are well adapted for study by the immersion method.

Hydrastine ($C_{21}H_{21}NO_6$) crystallizes in the orthorhombic system, usually consisting of colorless irregular fragments without definite habit. Many of these fragments extinguish sharply with crossed nicols while others remain partially extinguished under the same conditions when the microscope stage is revolved. However, only faint biaxial interference figures are revealed by the conoscopic examination. The significant refractive indices are: $\alpha = 1.550$ (occurring most frequently), $n_i = 1.734$ (but not the β value), $\gamma = >1.734$. Both ± 0.002. Only partial optical data have been reported heretofore (2).

Confirmatory Microchemical Tests for Hydrastine.—This test already has been described elsewhere (3, 4) and will only be briefly referred to here. On the addition of a drop of 5% hydrochloric acid and potassium ferrocyanide solution to the alkaloid, spherical masses of very minute needles, densely packed in the aggregates, will be observed. These circular groups *en masse* show a distinct polarization cross when examined with crossed nicols. *Hydrastine does not give the reddish-brown color with concentrated nitric acid.*

Berberine ($C_{20}H_{19}O_5N$) is distinctly yellow and usually composed of needles and rods, showing parallel extinction with crossed nicols, the sign of elongation being negative. Kley (5) has given partial microscopic-crystallographic data. The significant refractive indices as determined by the immersion method are: $\alpha = 1.490$ (frequently shown lengthwise), $n_i = 1.535$ (also frequently shown lengthwise), $\beta = 1.701$ (shown crosswise but not common), $\gamma = >1.734$ (shown crosswise). All ± 0.002.

Confirmatory Microchemical Test for Berberine.—When a drop of 5% hydrochloric acid is added (not in excess) to a small amount of berberine on an object slide, very small needles (probably the hydrochloride) are formed (6), these showing parallel extinction with crossed nicols, and negative elongation. Also, *berberine produces a distinct reddish brown color on the addition of concentrated nitric acid.*

SUMMARY

Attention has been called to a color reaction for berberine with concentrated nitric acid and its application to the characterization of *Hydrastis*. Microscopic-crystallographic properties of the principal alkaloids of *Hydrastis*, hydrastine, and berberine are also described, together with the significant microchemical tests and emphasizing certain features that can be observed with the polarizing microscope.

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A Simple Respirometer*

By THOMAS J. HALEY†

NUMEROUS methods for recording respiration in laboratory animals have been described. Jackson (1) has described a stethograph drum. Sollmann and Hanzlik (2) described a direct method employing a tambour connected to a tracheal cannula and an anesthetizing apparatus. Gaddum (3) has designed an elaborate apparatus which gives an excellent record of respiration. Pfeiffer and Moore (4) described a special cannula which records both the respiration and the pulmonary pulse. All of the above instruments have certain drawbacks which often result in the failure to obtain a record of the respiratory changes caused by various conditions or drugs. The Pfeiffer–Moore cannula gives very good results but the weight of the instrument may cause pressure effects upon other vital centers thus introducing artifacts obscuring the true results. To obtain accurate results and eliminate the above drawbacks the following instrument was designed.

Apparatus[1].—A piece of lucite tubing 2 inches by 4 inches was tapped in the center on both sides with a $^{5}/_{16}$-inch drill. Into these holes were inserted other pieces of lucite tubing $^{3}/_{8}$ inch by 3 inches. In order to make a welded joint these pieces were dipped in acetone before inserting and afterwards the area was coated with plastic cement. After the plastic had dried, two Acushnet M-2 valves and valve seats were forced into the ends of the large tube. See diagram (Fig. 1) for details. The valve seats must fit securely so that an airtight seal is obtained. The instrument has been constructed out of brass, copper, or glass tubing of the same sizes as the plastic but the latter was the most satisfactory because it was more readily worked, was unbreakable, and was more easily cleaned. The instrument can be built out of cardboard (mailing tube 2 inches in diameter) with glass side arms attached with plastic cement.

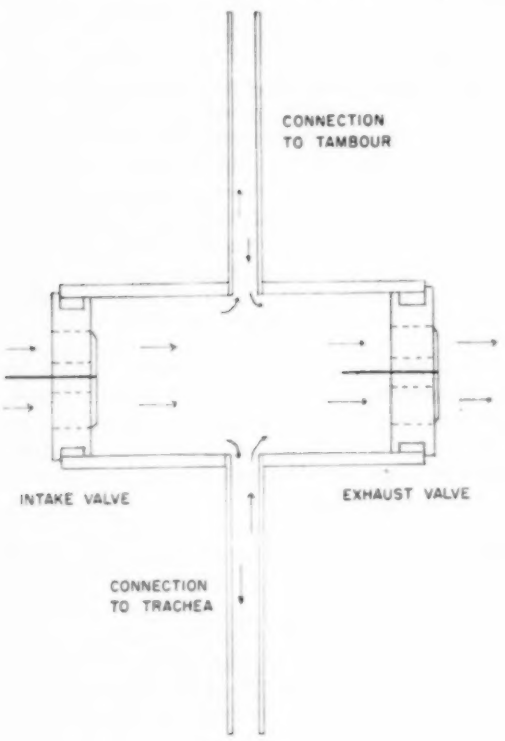

Figure 1

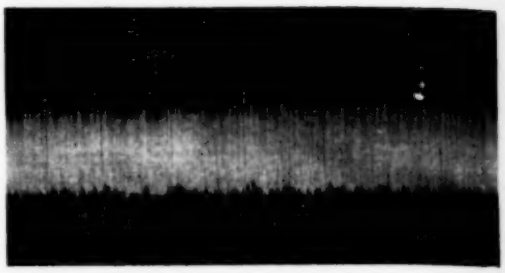

Fig. 2.—Respiration record: Rabbit under urethane-pentobarbital anesthesia.

Method.—In order to record respiration in the anesthetized animal the instrument is attached to the tracheal cannula with a short piece of rubber tubing and also in the same manner to the usual tambour. Inspiration is recorded by the downstroke of the tambour lever and expiration by the upstroke. Figure 2 shows a typical record obtained with a fixed dose anesthetic. When volatile anesthetics are used the intake valve of the instrument may be connected to the anesthetizing apparatus by a glass filter cup 2 inches in diameter. If the anesthesia becomes too deep the instrument is easily disconnected and the animal then breathes air.

The valves are not damaged by ether, chloroform, cyclopropane, divinyl ether, or other volatile anesthetics as long as only the vaporized and not the liquid agent is in contact with them (5).

The instrument can be adapted for recording the respiration of unanesthetized animals by molding an airtight face mask from sheet plastic and rubber.

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Book Reviews

United States Dispensatory. Twenty-fourth Edition By ARTHUR OSOL and GEORGE E. FARRAR. J. B. Lippincott Company, Philadelphia, Pa., 1947. xxii + 1928 pp. 27 x 18 cm. Price, $16.50.

The publication of a new edition of the United States Dispensatory is always an event of pharmaceutical interest. Comparison of the new 24th edition with the first edition, published one hundred and fifteen years ago, illustrates graphically and dramatically the progress made in drug therapy during the past century. The new 1947 edition, based largely upon U. S. P. XIII, N. F. VIII, the latest British Pharmacopœia and its seven addenda, and the 1947 New and Nonofficial Remedies, includes pharmaceutical and medical information concerning the drugs described in these books, as well as many unofficial drugs.

The portion of each monograph dealing with actions, therapeutic uses, and doses of drugs has, in nearly every instance, been rewritten, enlarged, and thoroughly modernized. It is regrettable that more interpretive information on official and unofficial tests and assays has not been included. In many assays, for example, a brief statement of the principles involved and the reasons for the steps in the procedures described would have greater reference value than the summaries of the assays which have been included. It is hoped that in subsequent editions greater attention may be given to supplying this kind of information, now that a good start in that direction has been made.

The new 24th edition of the Dispensatory is divided into five main divisions. In Part One monographs on drugs of the official compendia of the United States and Great Britain are included. These monographs are arranged in alphabetic sequence according to English titles. Each monograph furnishes information on the method of preparation or source of the drug discussed, official tests for strength, quality, purity, and identity in paraphrased and condensed form and a discussion of actions and uses. Registered trade-marks and some foreign names of these drugs are also included in many monographs.

In Part Two information relating to unofficial drugs is similarly presented. Illustrative of the material in this section, excellent résumés of the present status of antibiotic substances, antihistamine drugs, estrogenic drugs, and radium and radio activity may be cited.

Part Three is allocated to brief descriptions and entirely too brief interpretations of general tests and processes of the U. S. P. and N. F. Essential data on reagents and test solutions, volumetric solutions, and clinical laboratory reagents are also included.

Part Four, an entirely new section of the Dispensatory, deals exclusively with veterinary uses and doses of official and unofficial drugs. Tables of use in pharmacy are reproduced in Part Five.

The new 24th edition of the Dispensatory is well documented throughout, but the literature references cited in the discussions of uses and actions of drugs are particularly complete and significant.

The index is excellent and the binding and printing are adequate for a reference book of this type. When the magnitude of such a project as the revision and publication of the Dispensatory is considered the authors, editors, and publishers are to be complimented upon the apparent excellence of the finished product. The new Dispensatory should, when used in conjunction with other reference works, serve a useful purpose in any pharmaceutical library.

Microbial Antagonisms and Antibiotic Substances, by SELMAN A. WAKSMAN. Second edition. The Commonwealth Fund, New York, 1947. xi + 415 pp. 15 x 23.5 cm. Price, $4.00.

The subject of antibiotics has made such phenomenal progress in the last few years that less than three years after the publication of the first edition of Dr. Waksman's book [THIS JOURNAL, 34, 248 (1945)] a revision has been necessary. During this time penicillin has risen from a scarce and expensive product of mold metabolism to a known compound capable of existing in several similar chemical forms and producible with a relatively high yield. Streptomycin, which was little more than a laboratory curiosity in 1943, now shows great promise as a clinically effective chemotherapeutic agent. During the last three years many new antibiotics have been discovered, although few show much clinical effectiveness and none appears to be as spectacular as penicillin and streptomycin.

Dr. Waksman has introduced a wealth of new material on the formation, isolation, and utilization of antibiotics but has not increased the size of the book materially because many of the references of more historic interest have been eliminated.

The second edition of this reference book has been prepared with the same scholarly care which characterizes all of Dr. Waksman's undertakings.

Organic Analytical Reagents, by FRANK J. WELCHER. Volumes I and III. D. Van Nostrand Company, New York, 1947. xv + 442 pp. 14.5 x 23 cm. Price $7 series price; $8 individual price.

During the past several years there has been an increased interest in the use of organic reagents for analytical procedures, both in the detection and in the quantitative determination of many substances. Volume I of this series by Dr. Welcher will be of much interest to those who have occasion to refer to such methods. Although at least two books have been written in this field, they have approached it from the viewpoint of the substance to be detected or determined, while Dr. Welcher approaches it from the standpoint of the reagent. Furthermore, Dr. Welcher has made a serious attempt to completely cover the literature in this field and in fact states in the preface that "the information used in this book is taken from all publications appearing prior to January 1, 1946." Due to wartime conditions some of the data had to be taken from *Chemical Abstracts* rather than the original literature. Some indication of the thoroughness of the literature search can be seen from the fact that there are 315 literature references to be found under 8-Hydroxyquinoline.

The first few chapters are succinct accounts of the electronic theory of valence, coordination compounds, chelate compounds, and the effect of structure on solubility. This is followed by the reagents classified according to their chemical structure into hydrocarbons, alcohols, phenols, etc. Under each compound are given a formula, molecular weight, synonym, and reference to Beilstein. These are followed by a brief statement of the use of the compound in analytical chemistry and a description. Usually there appears a brief statement of the preparation of the compound. If the reagent in question is commonly available no details of its preparation are given. When the reagent is more rare more details concerning its preparation are given, accompanied by literature references.

This introductory material is followed by a more or less detailed account of the analytical uses for the reagent. Wherever possible specific directions for carrying out the procedure are given.

Volume III is divided into three parts: the heterocyclic nitrogen compounds, the oximes, and the acidic imino compounds. Like the first volume, it gives every evidence of meticulous care in its preparation.

The Indian Pharmacopoeial List 1946. Compiled and edited for the Government of India by a committee appointed by the Drugs Technical Advisory Board. Government of India Press, Calcutta, 1946. lx + 199 pp. 16 x 24 cm.

The purpose of the Indian Pharmacopœial List is to supplement, but not to replace, the British Pharmacopœia in India as a book of standards for drugs. In 1944 the government of India requested the Drugs Technical Advisory Board to compile a list of drugs which are not included in the British Pharmacopœia but which are of sufficient medicinal value to justify the establishment of suitable specifications for strength, quality, purity, and identity. In India more than 2000 indigenous drugs of vegetable, mineral, and animal origin are still being used and varying degrees of therapeutic claims are made for them. From this list, the committee, appointed to compile the Indian Pharmacopœial List, has chosen a limited number of botanical drugs considered to be of therapeutic value and has established official standards for them. In addition, a limited number of drugs included in the British Pharmacopœia have been made a part of the Indian list. Drugs in this group include only those that can be produced in India but which vary in minor respects from the specifications of the British Pharmacopœia.

Since the Indian Pharmacopœial List is intended only to supplement the British Pharmacopœia a large number of biological products and synthetic drugs has not been included. In these two groups, however, it is interesting to note the inclusion of Protein Hydrolysate Injection, Normal Human Serum, Tetrachloroethylene, Trichloroethylene, and Urea Stibamine.

Monographs in this book are arranged in alphabetical sequence according to Latin titles. The style employed is similar to that of the British Pharmacopœia. This book should be of particular value to those who are interested in a list of native Indian drugs which have not been used in the United States and to those who export drugs to India.

The printing and binding are of excellent quality and the committee of ten members appointed by the government of India should be complimented upon their efficiency in completing a project of this type in the short time allotted to the task.